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CANCER RESEARCH
A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH
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Changes in the Reducing Power of Serum or Plasma of Patients with Malignant Neoplastic Disease

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Many attempts have been made to demonstrate differential reactions in the serum of patients suffering from neoplastic diseases. In view of the relatively distinct alterations found in cellular metabolism with the advent of malignancy, particularly in regard to the oxidation-reduction reactions, it was felt that review of the properties of the blood from that point of view might prove fruitful. Further, there had been previous investigations along these lines which seemed to justify further study. Thus, Sehrt in 1929 (2) performed estimations of the oxidase activity of the blood using the Nadi reaction and reported an increased oxidase activity in the blood of 90 per cent of the cancer patients tested. Savignac and associates (1) reported an interesting correlation between the presence of malignancy and the reducing power of serum as tested by reaction with methylene blue.

Alterations in redox potential and reducing substances as found in tumor tissue and in the serum of tumor hosts are reviewed by Stern and Willheim (3). The authors point out that "evidence derived from work undertaken from various points of view appears to point clearly to an increased presence of reducing systems in malignant tumors." The papers reviewed by these authors failed to show any clear-cut differentiation in serum reducing power, although contradictory reports have been put forth from time to time.

It was the purpose of this investigation to evaluate alterations in the reducing power and throw some light on the significance of such variations.

METHOD

The evaluation of the reducing power was made by studying the interaction of plasma (or serum) with redox dyes. The following dyes were studied: methylene blue, brilliant cresyl blue, 2,6-dichlorobenzenoneindophenol, and cresyl violet. The potentials of these dyes as measured by the Weston electrometer were as follows: brilliant cresyl blue, 190 mv.; methylene blue, 220 mv.; cresyl violet, 295 mv.; 2,6-dichlorobenzenoneindophenol, 355 mv. It is to be noted that these are not Eo values, but are potentials as measured in the course of these experiments. These values are obtained by direct reading from the scale after the electrodes have been in contact with the solution tested for 7 minutes. The pH values of the mixtures of the dye and plasma were about 7.4 to 7.6. Of these it was found that brilliant cresyl blue and methylene blue gave significantly different reactions with plasma or serum from patients with and without neoplastic disease.

The technic consists of adding 0.2 cc. of a 0.1 per cent solution of methylene blue to 1 cc. of serum or plasma in a Kahn tube. A like amount (0.2 cc.) of a 0.1 per cent solution of brilliant cresyl blue is added to 1 cc. of serum or plasma in a second tube. After mixing, the tubes are immersed in a boiling water bath. A beaker served as a water bath so that changes in color could be seen with ease. The methylene blue used in this study was made by Mallinckrodt and differences in reaction were found with several other samples tested.

The tubes are watched for decolorization of the dyes. Thus, the tube containing the plasma and methylene blue is watched for complete decolorization of the dye and the time noted. In the case of the tube containing the brilliant cresyl blue, a slightly different technic is employed. Here the reaction is read at 10 minutes. Complete reduction of the brilliant cresyl blue results in a white or greyish-white color, while incomplete reduction leaves a lavender shade of varying intensity. The color is noted at 10 minutes and the tube then removed from the water bath and cooled under tap water. With cooling, characteristic alterations are seen to occur at the surface and in the mass of the coagulated plasma. These changes are of two main types: 1. surface—bluish-

green, mass—white or grey; 2. surface—blue-violet, mass—lavender. While a little experience is needed to familiarize the eye with these colors, they prove to be highly specific. Thus, plasma from a patient with malignant neoplastic disease is seen to be lavender after 10 minutes in the boiling water bath and to react on cooling with a violet surface and a lavender mass. On the other hand, complete reduction to a grey-white color is found in the non-malignant groups and on cooling, the surface becomes blue-green and the mass grey-white.

The time values found for the reduction of methylene blue by the plasma of patients with malignant neoplastic disease differed from the time values of patients with non-malignant neoplastic disease. In the majority of cases, the blood of patients suffering from malignant neoplastic diseases gave reducing times of 10 minutes or more as tested by this method. The blood of normal individuals showed the greatest concentration of values below 9 minutes, while the plasma of patients with non-malignant disease tended to give values below 10 minutes.

As will be shown below, various types of malignant growths tended to be associated with somewhat characteristic alterations in the reducing power of the plasma.

Between the range of 0.50 to 0.120 per cent, the potential of the methylene blue solution varies inversely with the concentration. On further dilution the slope flattens considerably. Thus, the following readings are obtained: 0.5 per cent, 150 mv.; 0.250 per cent, 187 mv.; 0.12 per cent, 220 mv.; 0.10 per cent, 225 mv.; 0.03 per cent, 225 mv. The reducing times of the 3 latter solutions measured with 1 plasma sample as a control vary greatly in spite of the proximity of the potential values; *viz.*, 0.03 per cent, 6.5 minutes; 0.10 per cent, 9 minutes; 0.12 per cent, 15 minutes.

The calibration of a new lot of 0.1 per cent methylene blue is accomplished by comparing the time needed for complete decolorization of a control plasma sample with the time needed with the standard solution. If the time value of the new lot is prolonged, the lot should be diluted with distilled water. If the time value is low, the solution should be concentrated by evaporation in an open vessel. It follows that solutions should be carefully stoppered when not in use.

The standardization of the brilliant cresyl blue solution is done in the same manner with one exception. This exception is the end point. With brilliant cresyl blue, the end point is not a complete decolorization phenomenon but a definite color change, and as a result, this solution must be standardized with two plasma samples—one from a patient with malignant disease and another from a normal individual. The end points of the control samples are those described above.

RESULTS

Analysis of the results obtained with methylene blue may be made along several lines: 1. the spread of values with (a) normal controls, (b) non-malignant disease, and (c) malignant disease; 2. study of the alterations with the various types of malignant tumors; 3. the effect of therapy on the reducing power of the plasma.

In the studies with brilliant cresyl blue, the reactions as studied here are of the all-or-none type, plasma from patients with malignant tumors showing incomplete reduction and that from patients with non-malignant processes resulting in complete reduction. Thus, the final evaluation of the serodiagnostic value of the reducing power of the blood rests on the reaction with both dyes.

TABLE I: METHYLENE BLUE REDUCTION TIME FOR NORMAL BLOOD PLASMA

Time min.	No. of cases	Per cent of total
6-7	6	8
7-8	14	21
8-9	32	41
9-10	19	25
10-10.5	4	4
10.5-11	1	1
	76	100

* The interaction with brilliant cresyl blue gave complete reduction in all these cases; *viz.*, surface—blue-green, mass— gray-white.

When the plasma of presumably healthy individuals is tested by this method, it is found that the great majority shows complete reduction of the methylene blue before 10 minutes (Table I). In a few cases, the time values are longer, but even in these cases complete reduction of brilliant cresyl blue has been found, so that differentiation is possible. It might be mentioned that it is not necessary to limit tests to individuals who have fasted, although in some cases a fatty plasma makes it more difficult to evaluate color changes with the brilliant cresyl blue. The reactions obtained with the plasma of patients suffering from a variety of non-neoplastic diseases were for the most part similar to those obtained in the control group (Table II).

TABLE II: NON-NEOPLASTIC DISEASE, METHYLENE BLUE REDUCTION

Diagnosis	No. of cases	False interpret.*	Av. Time min.
Adenopathy, infectious	2		6.5
Anorexia	1		8.5
Appendicitis	1		8.5
Arthritis	1		8.5
Arteriosclerotic heart disease	5		8.2
Asthma, bronchial	3		8.6
Bronchiectasis	1		9.5
Beck sarcoid	1		8.5
Bronchial cyst	1		9.0
Cardiac decompensation	1		7.0
Carbuncle	1		7.0
Cardiospasm	1		10.0
Cirrhosis, hepatic	6	3	10.9
Cholecystitis	1		8.0
Colitis	2		9.0
Coronary insufficiency	2		8.5
Cystitis	2		8.5
Dermatitis, subacute and chronic	1		8.5
Diabetes; HCVD	6		8.3
Endocarditis, subacute bacterial	1		8.5
Endometritis	2		9.0
Fever, undetermined origin	1		9.0
Gastritis	3		9.6
Granuloma, fungus	1		9.0
Hemorrhoids	1		6.0
Hepatosplenomegaly	2		8.0
Hypertensive cardiovascular disease	8	1	9.1
Hypertension, essential	1		9.0
Infectious mononucleosis	1		9.0
Lues	1		7.0
Lymphedema	1		10.0
Malnutrition	1		9.0
Menopausal syndrome	2		7.7
Muscular dystrophy	1		9.0
Nephritis	1		9.0
Nevus, pigmented	1		8.5
Nucleus pulposus	2		8.0
Oophorectomy	1		9.0
Paget's disease	1		8.0
Parkinsonism	1		8.0
Pernicious anemia	5	1	9.2
Pharynitis, streptococcus	1		9.0
Pneumonia	5		9.4
Pregnancy, 5 months	3		10.0
Pregnancy, 8-9 months	2	2	14.0
Prostatic hypertrophy	2		9.5
Prostatitis	1		9.0
Psychosis, senile	1		9.0
Pyelitis	2	1	10.5
Rheumatic heart disease	2		10.0
Tuberculosis	7		8.8
Ulcer, peptic	5	1	10.0
Ulcer, trophic, leg	1		7.0
Vaginitis	1		8.0
Weber-Christian syndrome	1		10.5
Totals	112	9	

* The reactions with methylene blue and brilliant cresyl blue were indistinguishable from those obtained with blood from patients with neoplastic diseases. These determinations are included in the average time values.

However, it should be noted that some samples of plasma showed prolonged reducing time with the methylene blue and incomplete reduction of the brilliant cresyl blue. These divergencies occurred particularly in cases where there was hypoproteinemia, *viz.*, cirrhosis. Thus, in the presence of a marked hypoproteinemia, less than 5 per cent, it is possible to obtain false positive results.

The presence of non-malignant neoplasia fails to induce significant variations in the plasma of patients as measured by this method (Table III). Thus, plasma of patients with benign tumors as well as such diseases as chronic leukemia, multiple myeloma, and polycythemia reacts similarly to the plasma of the controls.

TABLE III: NEOPLASIA, NON-MALIGNANT. METHYLENE BLUE REDUCTION

Diagnosis	No. of cases	False†	Av. time min.
Papilloma, bladder	1		9.5
Breast mass, non-malignant	4		8.8
Giant Cell Tumor	1		9.5
Lipoma	2		8.5
Leukoplakia	2		6.5
Leukemia, chronic, myelog.	4	1	9.8
Leukemia, chronic, lymphatic	3		9.5
Leukemia, chronic, lymphatic, (marked ascites)	1	1	18.0
Multiple myeloma	5		9.2
Polycythemia	2		10.0
Xanthoma	1		9.0
Totals	26	2	

* These cases give "non-malignant type" type of reaction with brilliant cresyl blue.

† The reactions with methylene blue and brilliant cresyl blue were indistinguishable from those obtained with blood from patients with malignant neoplastic diseases. These determinations are included in the average time values.

The plasma of patients suffering from malignant neoplasia showed characteristic increase in methylene blue reduction time as well as incomplete reduction of brilliant cresyl blue (Table IV). In addition, there was a much greater spread of values. There tended to be somewhat characteristic time values for the methylene blue reduction with plasma of patients with the various types of malignant conditions. Thus, plasma from patients with ovarian and cervical carcinomas gave long methylene blue reduction times.

TABLE IV: ACTIVE MALIGNANT NEOPLASIA. METHYLENE BLUE REDUCTION

Site of origin	No. of cases	False*	Av. time min.
Bladder, adenocarcionoma	2		11.2
Breast, duct cell carcinoma	22	4	12.4
Carcinomatosis	1		10.5
Cecum, adenocarcinoma	1		12.5

Cervix, squamous cell carcinoma	28	4	15.0
Chondrosarcoma	1	1	7.0
Chorioepithelioma	1		13.0
Colon adeno-carcinoma	12	3	13.0
Esophagus, squamous cell carcinoma	4	1	14.0
Ewing's tumor	1		12.0
Glioblastoma multiforme	1	1	8.0
Hemangiosarcoma	1	1	8.0
Hodgkin's disease	15	5	13.5
Intestine, adenocarcinoma	1		20.0
Hypernephroma	2		10.5
Larynx, squamous cell carcinoma	9	1	12.5
Lung, bronchogenic carc.	19	2	13.0
Lymphoepithelioma	1		11.0
Lymphosarcoma	8		14.6
Leukemia, acute, lymphoblastic	5		12.4
Leukemia, acute, monoblastic	2		13.5
Leukemia, acute, myeloblastic	11	3	11.5
Lip, epithelioma	2	1	15.0
Melanoma	2	1	15.0
Mouth, epithelioma	3	1	12.5
Nasopharynx, transitional cell carcinoma	1		15.0
Ovary, adenocarcinoma	8		15.0
Pancreas, adenocarcinoma	1		11.5
Prostate, adenocarcinoma	11	2	12.7
Rectosigmoid, adenocarcinoma	5		13.4
Sarcoma	4		14.7
Stomach, adenocarcinoma	16	2	14.0
Skin, basal cell carcinoma	8	4	12.4
Skin, epithelioma	5	3	10.2
Testes, embryonal cell carcinoma	3	3	8.5
Tongue, epithelioma	2	2	9.0
Tonsil, squamous cell carcinoma	4		11.8
Thyroid, adenocarcinoma	6	3	11.3
Uterus, fundus, adenocarcinoma	6		13.3
Vulva, squamous cell carcinoma	2		11.0
Totals	237	48	

* The reactions with methylene blue and brilliant cresyl blue were indistinguishable from those obtained from presumably healthy individuals. These values are *not* included in the averages.

It should also be noted that the occurrence of discrepancies between the clinico-pathologic diagnosis and the type of reducing reaction was greater here than in the previous groups. Thus, a false negative reaction was somewhat more common than a false positive. Although the failure to detect alterations in some of these cases is not explainable, in others, previous or coincident therapy, either surgery or radiation, may have caused reversal of reactions (Table V). The ability of therapeutic measures to alter these reactions is of particular importance for it provides an objective index of the efficacy of a therapeutic procedure. Thus, examination of Table V reveals how therapeutic procedure may alter the reactions under discussion.

TABLE V: EFFECT OF THERAPY ON METHYLENE BLUE REDUCTION TIME

Carcinoma of	Therapy	M.B. Red. T.*	Impression†
Breast	Mastectomy, 2 yrs. ago	10	n.m.‡
Breast	Mastectomy, 1 mo. ago	9	n.m.
Breast	Mastectomy, 2 wks. ago	8	n.m.
	(local lesion)	6	n.m.
Breast	Mastectomy, 3 yrs. ago		
Breast	Mastectomy, 7 yrs. ago	9	n.m.
Chorioepithelioma	Hysterectomy, 1 yr. ago clinically well, A-Z neg.	7	n.m.
Cervix			
Apr. 25, 1946		20	m.§
May 1, 1946	X-ray, pelvic adenectomy		
Apr. 29, 1946		16	m.
May 20, 1946	clinically improved	11	m.
Cervix	Extensive x-ray	9	n.m.
Cervix	Extensive x-ray	8.5	n.m.
Prostate	Postop., estrogens acid phosphatase 22	9	n.m.
Rectosigmoid			
Apr. 10, 1946	Preop.	15	m.
Apr. 23, 1946	Postop. 3 days	12	m.
Apr. 26, 1946	Postop. 6 days (pt. expired)	12	m.
Rectum			
Aug. 12, 1946	Small local lesion	9	m.
Aug. 21, 1946	Postop. 2 days	14	m.
Aug. 24, 1946	Postop. 5 days	11	m.
Uterus, fundus	Postop. 5 years symptom-free	8.5	n.m.

*M.B. Red. T.=methylene blue reducing time.
†The impressions are formed on the basis of the methylene blue reducing time and the reaction to the brilliant cresyl blue.
‡n.m.=non-malignant.
§ malignant.

The distinction between the results obtained with the various classes of sera is illustrated in Figs. 1 and 2. Although the different groups possess a zone of overlap in the methylene blue reduction time, it is possible in most cases to make correct differentiation by virtue of the differences in reaction of the brilliant cresyl blue.

The ratio of accuracy in diagnosis as found in this series is recorded in Table VI. It should be noted again that the tendency for a false negative is somewhat greater than for a false positive. This is particularly true if one eliminates cases of marked hyproteinemia which tend to give false positive reactions. This does not mean that this reaction is a direct measure of the protein content of the plasma, for no such correlation is found when the plasma proteins are measured.

TABLE VI: SUMMARY OF DIAGNOSTIC TESTS AND ACCURACY RATIO

Diagnosis	No. of cases	No. false	Accuracy ratio	
Control	76	0	76/ 76	203/214
Non-neoplastic diseases	112	9	103/112	
Non-malignant neoplasia	26	2	24/ 26	
Active malignancy	237	48	189/237	
Totals	451	59	392/451	

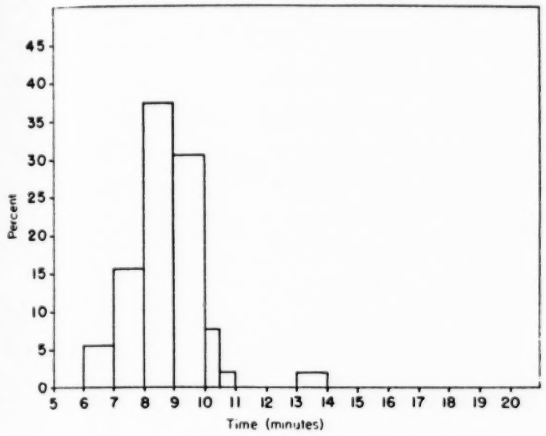

Fig 1 - Average time distribution of methylene blue reduction by plasma of normal individuals and patients with non-neuplastic diseases

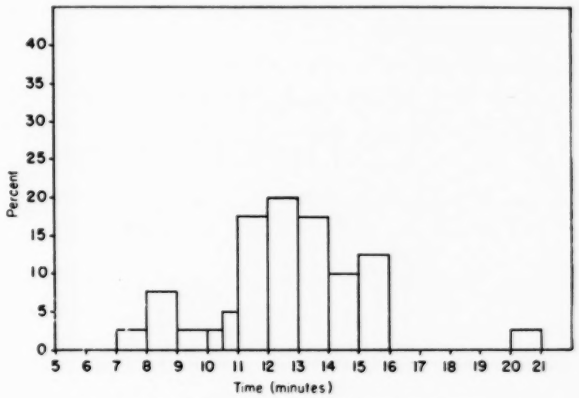

Fig 2 - Average time distribution of methylene blue reduction by plasma of patients with malignant neoplastic diseases.

DISCUSSION

The results obtained in this investigation add additional evidence to the concept that malignant neoplasms induce changes in the host at distant sites. In this case, it would appear that the reaction of reduction of methylene blue and brilliant cresyl blue depends on an alteration in the reducing groups of a protein fraction, probably the albumin fraction.

In the presence of a marked hypoproteinemia (plasma protein of 5 per cent or less) the test is invalid for it will tend to give false positive reactions. Thus, false positive reactions were obtained in some cirrhotic patients and, in a case of chronic leukemia, with marked recurrent ascites. These conclusions are in accord with those of Savignac and associates (1).

SUMMARY

1. Determination of the reducing power of plasma (or serum) was made by the use of the redox dyes, brilliant cresyl blue, and methylene blue.

2. Plasma of patients with malignant diseases tended to have a lowered reducing power and could be differentiated with a high degree of accuracy.

3. Adequate therapy reversed the characteristic alterations in reducing power of the plasma of patients with malignant neoplastic diseases.

4. The various malignant neoplasias gave varied degrees of alteration of the reducing power.

ACKNOWLEDGMENT

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Activity of Catheptic Enzymes in *p*-Dimethylaminoazobenzene Hepatomas*

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(Received for publication November 7, 1946)

Greenstein (12) has pointed out that the enzymatic activity in tumor tissue in most cases is less than that found in its normal counterpart. One of the few exceptions to this generalization is the activity of tumor cathepsin, as reported by Maver and associates (19), in experiments using hemaglobin as a substrate.

The present experiments were designed to compare the catheptic activity of rat hepatomas with the activity of control livers. In place of protein substrates, peptide substrates first employed by Bergmann and Fruton (3, 4) have been used, in order to obtain more detailed information on the types of peptide bonds involved in the enzymatic hydrolyses.

The results indicate that activity of the catheptic enzyme responsible for the hydrolysis of benzoyl-*l*-argininamide, termed by Bergmann a trypsinase, was increased in the hepatoma, under the experimental conditions used.

Since the completion of this work, Greenstein and Leuthardt (13) have reported an increased benzoylargininamidase activity in the transplanted rat hepatoma 31, but not in the primary rat hepatoma, as compared with various normal controls. Their experiments were carried out at pH 6.9, and it appeared doubtful whether the enzyme involved was the same as that which hydrolyzed benzoylargininamide at pH 4.6.[1]

METHODS

Fifteen Sherman male rats, 5 months of age, were allowed to consume diet 11 of Miller and collaborators (20), containing 0.06 per cent of *p*-dimethylaminoazobenzene (butter yellow). The animals were placed in two cages, and the average daily consumption of food was measured. Fifteen control rats of the same sex, age and weight were maintained on an adequate stock.[2] From the start of the experiment, rats (both *p*-dimethylaminoazobenzene diet and control) were killed at intervals by decapitation, and as much blood as possible was allowed to flow out from the severed carotid arteries. Autopsies were performed, and sections of various organs were preserved in Zenker's solution or 10 per cent formalin for histological study. Livers were weighed, minced finely with scissors, frozen immediately, and lyophilized according to a modification of the technic of Flosdorf and Mudd (7). The dry tissue was weighed, ground to a fine powder, and stored over phosphorus pentoxide in a vacuum desiccator at 3° C. Enzymatic determinations were, with a single exception, carried out on aliquots within 8 days. A normal control rat was always killed at the same time as one on the *p*-dimethylaminoazobenzene diet. Both livers were prepared in the same way, and enzyme studies were carried out on them at the same time.

In order to obtain regenerating liver, partial hepatectomies (14) were performed on 5 Sherman control rats. The portions of liver removed were treated as above and served as controls. During the second 24 hours following partial hepatectomy, the rate of regeneration of liver has been found to be maximal (5). After approximately 30 hours, therefore, the rats were killed and the livers treated as described above.

* This is reprint No. 625 of the Cancer Commission of Harvard University. This work was aided by a grant from The Donner Foundation, Incorporated.

Presented in part at the meeting of the American Association for Cancer Researh, Inc., at Atlantic City, New Jersey, March 12, 1946 (28).

[1] In a personal communication, Greenstein has recently informed the authors that he also finds increased activity toward benzoylargininamide in the primary hepatoma, when the experiments are carried out in the pH range between 5.0 and 6.5.

[2] Echelman's Red Rose Dog and Puppy Food.

Fetal livers were obtained from 6 Sprague-Dawley rats at term. Livers of fetuses from the same mother were pooled, then weighed and lyophilized.

Enzymes.—The following substrates were used in comparisons between control livers and hepatomas: benzoyl-*l*-argininamide, *dl*-leucylglycine, carbobenzoxy-*l*-glutamyl-*l*-tyrosine, and (in a few experiments) carbobenzoxylglycyl-*l*-phenylalanine.[3]

The rate of hydrolysis was determined by the method of Grassmann and Heyde (10). Enzyme solutions were prepared in a manner similar to methods previously described (18, 27); 900 mgm. of the dried liver were suspended with 10 cc. of 50 per cent glycerol in a tissue homogenizer (21) for 3 minutes. The suspension was centrifuged for 3 minutes at 2,400 r. p. m., and the supernatant was filtered through a thin layer of Pyrex glass wool, to remove bits of liver powder that remained at the surface. The filtrate was kept at 0° C. and aliquots were used within an hour for enzyme experiments. This procedure has been shown to provide reproducible enzymatic activities on separate aliquots of the same tissue powder (27). Whereas aqueous extracts of lyophilized liver gave a large autolysis blank, 50 per cent glycerol extracts gave a small blank.

The composition of the test solution was as follows: 0.6 cc. enzyme solution, 0.2 cc. of 0.5 *M* citrate buffer (Sorensen's citrate—NaOH mixture), 0.2 cc. of 0.1 *M* cysteine hydrochloride, 1.0 cc. of 0.1 *M* substrate (0.12 *M* for *dl*-leucylglycine), and 0.01 cc. of toluene. Incubation was carried out in a 2.5 cc. volumetric flask at 40° C. in the presence of air. All reactions were run at a pH between 5.0 and 5.4. Initial and final pH determinations were performed by means of a glass electrode pH meter, and were found, with but rare exceptions, to agree within 0.1 pH units. The cysteine solution was freshly prepared before use. Although catheptic activity was present without the addition of cysteine, the precaution of adding sufficient cysteine for maximal activation was used in order to minimize the possibility that enzymatic activity differences might reflect differences in activator concentration. Little or no autolysis was present in the controls, when substrate was incubated separately from the remainder of the test solution, and aliquots of these two fractions of the test solution were mixed just prior to titration. The enzyme-containing solution contained 30 per cent glycerol. The majority of the autolysis blanks were performed in this way. In certain later experiments water was substituted for the substrate solution in the autolysis control, which was then incubated. There was invariably a small amount of autolysis present under these conditions.

The presence of activator (natural plus added cysteine) in the experiments in which carbobenzoxy-*l*-glutamyl-*l*-tyrosine was used as a substrate introduces the possibility that a carboxypeptidase (cathepsin IV) is contributing to the hydrolysis of this substrate. In order to eliminate this possibility, it would be necessary to dialyze the glycerol extracts, thus introducing a variable error due to enzyme denaturation during dialysis. Overnight dialysis of 50 per cent glycerol extracts of liver powder against 50 per cent glycerol at 3° C. has resulted in a loss of one-half to two-thirds of the original activity.

The catheptic enzyme which hydrolyzes carbobenzoxy-*l*-glutamyl-*l*-tyrosine has been found to be more heat-labile than other catheptic enzymes (8). Comparison of reaction rates have, therefore, been based on data obtained from the initial reading only.

The influence of enzyme concentration on activity is illustrated in Table I. It will be observed that the activity per mgm. of total nitrogen, C_D, changed very little with dilution of the enzyme-containing solution. Experiments were arranged, in dealing with both normal and malignant livers, so that the total nitrogen concentrations of the test solutions fell between 1 and 2.5 mgm. of nitrogen per cc. of test solution. The average total nitrogen concentration of 50 per cent glycerol extracts of hepatomas and pre-hepatomas was practically the same (1.72 and 1.63 mgm. of nitrogen per cc. of test solution), but this value was somewhat higher for control livers (2.17 mgm. of nitrogen per cc. of test solution).

TABLE I: RELATION OF ENZYME CONCENTRATION TO ACTIVITY PER MGM. TOTAL NITROGEN

Nitrogen conc., mgm. per cc.	$K^*4 \times 10^9$	$C†4 \times 10^9$
2.43	184	76
1.22	85	70
0.61	44	72

Substrate benzoyl-*l*-argininamide
pH 5.1
T 40°
Cysteine 0.01 M
Citrate buffer 0.05 M.
Test solution contained 15 per cent glycerol
* K_D=-dS/dt (26)=moles hydrolyzed per minute per cc. test solution.
† C_D=K_D/mgm. nitrogen per cc. of test solution (exclusive of substrate nitrogen). Moles hydrolyzed per minute per mgm. nitrogen.

[3] The authors wish to express thanks to Dr. G. W. Irving, Jr. and to the late Dr. Max Bergmann for supplies of carbobenzoxy-*l*-glutamyl-*l*-tyrosine and carbobenzoxy-glycyl-*l*- phenylalanine. The other substates were obtained through the courtesy of Professor M. S. Dunn and the Amino Acid Manufactures, Los Angeles, California.

A series of 20 aliquots of a test solution containing *dl*-leucylglycine, buffer, activator, and 50 per cent glycerol extract of liver was pipetted into titration flasks. Ten aliquots were titrated immediately, the reaction being stopped by addition of alcohol. Ten other aliquots were frozen, thawed out individually at intervals during the course of the day, and titrated. A combination of these conditions simulated those under which experiments were conducted, and tested the visual and other errors involved in the endpoint. For an average titration figure for the whole series of 1.79 cc., the standard deviation was 0.028 cc. Under similar conditions, 100 per cent hydrolysis of a substrate would give a titration difference of 0.66 cc.

In experiments in which purified cathepsins, prepared by ammonium sulfate fractionations, were used, Irving, Fruton, and Bergmann (15) found that the reactions followed first order kinetics, in which $K=1/t \log a/a-x$. As the substrate concentration was varied, the initial reaction velocity changed correspondingly, but the first order K remained unchanged. Whereas these investigators employed enzyme preparations of relatively high specific activity, in the present experiments less purified enzyme preparations were used. Table II indicates that the *initial* reaction velocity of the enzymes hydrolyzing *l*-leucylglycine and carbobenzoxy-*l*-glutamyl-*l*-tyrosine was independent of the substrate concentration in the range (0.05 molar) in which the experiments were carried out.

TABLE II: EFFECT OF INITIAL CONCENTRATION OF SUBSTRATE ON REACTION VELOCITY

Substrate	Substrate conc. mole $\times 10^6$/cc.	Enzyme conc. mgm. nitrogen/cc.	Time, min.	Per cent hydrolysis	$K_D \times 10^3$
l-Leucyglycine	125	2.22	200	8.9	56
	38		200	32	60
	25		200	48	60
Carbobenzoxy-*l*-glutamyl-*l*-tyrosine*	94	0.98	10	0.89	84
	47		10	1.9	89
	12		10	7.2	84
Benzoyl-*l*-argininamide	75	2.18	170	23	102
	50		170	32	94
	38		170	40	89
	21		170	69	85

Hydrolysis followed by a manometric method (29). This method depends on the ability of a *Streptococcus faecalis*, strain R, decarboxylase to act on free tryosine at pH 5.5, but not on tyrosine as present in the peptide linkage carbobenzoxy-*l*-glytamyl-*l*-tyrosine. Other experimental details as in Table I.

In the case of the enzyme hydrolyzing benzoyl-*l*-argininamide, the hydrolytic rate fell somewhat with decrease in initial substrate concentration. For all three substrates, the initial portion of the reactions was found to correspond more closely to zero order ($K_d = -dS/dt$) than to first order kinetics. Calculations of results for benzoyl-*l*-argininamide were also made on the assumption that first order reaction kinetics were being followed. This precaution was taken to find out whether the statistical significance of the differences observed depended on the method of calculation. The differences, as expressed in Tables IV to VIII, were nevertheless found still to be significant.

Total nitrogen was determined by the micro-Kjeldahl modification of Wagner (25). The nonprotein nitrogen fraction was found to be a small, fairly constant part of the total liver nitrogen (control livers 0.61, 0.54, 0.50; hepatomas 0.79, 0.81, 0.88 per cent of total nitrogen). Enzymatic activity has therefore been related to the total nitrogen (exclusive of substrate nitrogen) rather than to the protein nitrogen.

Boiled liver extracts (kochsafts) were prepared from lyophilized normal liver and hepatoma powders as follows: 1,800 mgm. of powder pooled from several separate livers was ground in a mortar for 5 minutes with 20 cc. of glass-distilled water. The suspension was boiled for 3 minutes, reground in a mortar, boiled again for 5 minutes, centrifuged, and the supernatant was evaporated to one-sixth of its volume. The final extracts were tested for their activating powers as described later.

Cholesterol.—Total and free cholesterol determinations on the dried livers were carried out according to Sperry's modification (24) of the Schoenheimer-Sperry method for the determination of cholesterol. An alcoholic solution of digitonin as recommended by Sobel (23) was, however, substituted for an aqueous one.

Lipid.—The total lipids of the livers were extracted by means of an alcohol-ether solution. The extract was evaporated, re-extracted with hot chloroform, filtered, and dried to constant weight.

Histology of tumors.—The tumors were all parenchymal cell hepatomas types I and II, similar to those described by Edwards and White (6). In 3 out of 6 cases, metastases to regional mesenteric lymph nodes were present. In one case (rat 10) a small area of bile duct carcinoma was present in addition to the parenchymal cell tumor.

The term "pre-hepatoma" has been used in these studies to characterize the livers of animals on the *p*-dimethylaminoazobenzene diet sacrificed prior to the development of hepatomas. This group, therefore, serves as a type of control differing from the hepatoma group only by length of time on the diet.

RESULTS

Specificity studies on dl-leucylglycine splitting enzyme.—The hydrolysis of *dl*-leucylglycine stopped at 50 per cent splitting of the *dl*-substrate, suggesting that only one component was hydrolyzable. Since *l*-leucylglycine was hydrolyzed, it may be inferred that the *d*-form is not split. The comparative rate of hydrolysis of the various substrates, expressed as $K_D \times 10^{-9}$, was as follows: *l*-leucylglycine 69, *dl*-leucylglycine[3] 48, *dl*-leucinamide[4] 136, carbobenzoxy-*l*-leucylglycylglycine 160, *l*-leucylglycylglycine 162, *dl*-alanylglycine[3] 58. *dl*-Leucinamide is hydrolyzed by an aminopeptidase, but carbobenzoxy-*l*-leucylglycylglycine cannot be split by an aminopeptidase, since there is no free amino group. This latter substrate may be split by a carboxypeptidase or an endopeptidase. It is possible, therefore, that the *dl*-leucylglycine is hydrolyzed by more than one enzyme, under the experimental conditions used.

Effect of activators.—Twenty cubic centimeters of a 50 per cent glycerol extract of normal liver, prepared as stated previously, were dialyzed for 48 hours at 3° C. against 4 changes of 500 cc. portions of 50 per cent glycerol. A dialyzed extract of hepatoma was prepared similarly. The ability of these dialyzed extracts to hydrolyze *dl*-leucylglycine at pH 5 was tested under the following conditions: (A) without added activators; (B) with (a) 0.01 *M* cysteine (b) 0.001 *M* manganese chloride, (c) in the presence of (a) plus (b), (d) with "kochsaft" from normal liver, (e) with kochsaft from hepatoma, (f) with 0.01 *M* cysteine in addition to each separate kochsaft. The results of these experiments indicated that the dialyzed enzyme alone was only slightly active, and was maximally activated by cysteine. The kochsafts had no appreciable activating effect alone. In combination with cysteine they did not enhance the activation provided by cysteine alone. No appreciable difference in the activation ability of the liver and hepatoma was observed.

Manganese had no activating effect at pH 5, although in agreement with the findings of others (2, 22) a leucylglycine-splitting enzyme activatable by manganese was found at pH 7.5.

Comparison of control livers with hepatomas.—Table III is a summary of various changes in the

[4] Calculated for hydrolysis of one optical isomer.

TABLE III: SUMMARY OF VARIOUS CHANGES OCCURRING IN HEPATOMAS AS COMPARED WITH PRE-HEPATOMAS AND CONTROL LIVERS

			LIVER									
Rat no.	Days on diet	Weight gm.	Histology	Wet weight gm.	Weight dry / ×100 wet	Total nitrogen, mgm.	Nitrogen per 100 mgm. dry weight, mgm.	Total lipid % dry wt.	Cholesterol mgm./100 mgm. dry weight: Total,	Free	Combined	$C_D \times 10^9$ BAA*
4	20	248	pre-hepatoma	7.6	30	214	9.26	26.2	0.83	0.20	0.63	77
15	32	268	"	7.8	33	242	9.70	23.6	1.40	0.30	1.10	63
11	32	246	"	8.6	35	240	8.27	36.2	1.29	0.42	0.87	40
2	116	225	"	14.6	33	344	7.27	43.3	1.89	0.55	1.34	58
3	116	200	"	10.0	34	219	6 45	43.5	2.36	0.45	1.91	85
6	116	200	"	10.0	34	228	7.14	38.3	2.25	0.46	1.79	94
12	180	230	hepatoma	30.3	26	765	9.57	24.3	1.93	0.68	1.25	110
10	187	205	"	35.2	20	714	10.4	18.9	1.74	1.02	0.72	110
9	193	258	"	52.5	21	1100	10.0	18.5	2.47	1.19	1.28	94
8	201	250	"	35.0	24	685	8.25	20.0	2.06	0.90	1.16	130
7	205	234	"	16.5	33	414	7.66	36.6	2.42	0.55	1.87	120
14	205	246	"	29.2	28	632	7.70	33.6	2.95	0.73	2.19	140
16		293	normal	7.6	30	281	12 2	13.1	0.90	0.71	0.19	65
17		285	"	7.7	34	312	12.0	9.1	0.88	0.76	0.12	55
24		245	"	6.3	37	272	11.8	13.0	0.81	0.73	0.08	48
27		330	"	10.3	32	385	11.0	11.9	0.62	0.50	0.12	35
20		285	"	8.0	28	263	11.6	15.1	0.77	0.66	0.11	39
23		292	"	8.3	32	299	11.4	13.9	0.70	0.57	0.13	44
30		338	"	11.8	31	385	10.4	13.1	0.77	0.56	0.21	61
26		288	"	10.3	28	322	11.1	11.8	0.74	0.60	0.14	67
Averages:												
Pre-hepatomas	231			9.7†	33†	248†	8 02	35.2	1.67	0.40†	1.27	69†
Hepatomas	236			33.1	25	718	8.93	25.4	2.26	0.84	1.41	120
Normal controls	294			8.9†	32†	315†	11.4†	12.6†	0.77†	0.64‡	0.14†	52†

* The abbreviation BAA refers to benzoyl-*l*-argininamide.
†Difference from hepatoma significant (1).
‡ Difference from hepatoma probably significant.

pre-hepatomas, hepatomas, and normal control livers.

Attention may be called to several points. The hepatomas are huge livers with high total lipid, cholesterol, and water concentration as compared with the normal controls. These latter 2 observations have previously been reported (12, 17). The activity per mgm. nitrogen of the cathepsin responsible for the hydrolysis of benzoyl-*l*-argininamide is significantly increased in the hepatoma (Table IV).

TABLE IV: COMPARISON OF ENZYMATIC ACTIVITIES, PER MGM. OF NITROGEN, OF HEPATOMAS WITH VARIOUS TYPES OF CONTROL LIVERS, USING THE SUBSTRATE BENZOYL-*l*-ARGININAMIDE*

Moles × 10^9 of substrate split per minute per mgm. total nitrogen
C_D pH 5.19-5.40

Control		Regenerating	Fetal	Pre-hepatoma	Hepatoma
65	67	49	55	77	110
55	37	44	59	63	110
48	37	48	65	40	94
35	35	49	57	58	130
39	31	50	45	85	120
44	48	—	53	94	140
61		48 ± 2.1	—	—	—
			56 ± 6.1	69 ± 17	120 ± 17
46 ± 16†					
p‡		<.01	<.01	<.01	<.01

* Each figure represents the average of 3 separate determinations obtained in an experiment on a single liver. In the case of the fetal livers, 8-10 livers were pooled for each experiment.
† Standard deviation.
‡ Probability that the difference from the hepatoma is due to chance (1).

TABLE V: COMPARISON OF AVERAGES OF ENZYMATIC ACTIVITIES PER MGM. DRY LIVER, OF HEPATOMAS WITH VARIOUS TYPES OF CONTROL LIVERS, USING 3 SUBSTRATES

Moles × 10^9 of substrate split per minute
pH 5.19-5.43

Substrate	BAA*		CGT		LG	
Type of enzyme	trypsinase†	n	pepsinase	n	amino-peptidase	n
Control	3.8‡ ± 1.2	13	5.9 ± 2.1	5	2.6 ± 1.6	8
Regenerating	3.6‡ ± 1.7	5				
Fetal	4.6‡ ± 1.2	6				
Pre-hepatoma	4.1‡ ± 1.2	6			2.2 ± 1.2	6
Hepatoma	7.6 ± 1.2	6	7.4 ± 2.8	6	3.4 ± .91	6

* Meaning of abbreviations as follows:
n=Number of animals on which individual determinations were made.
BAA=benzoyl-*l*-argininamide.
GCT=carbobenzoxy-*l*-glutamyl-*l*-tyrosine.
LG=*dl*-leucylglycine (activity calculated on basis or hydrolysis of *l* form only.)
† The enzyme classification is that used by Bergmann (3). Greenberg and Winnick (11) suggest that this enzyme be termed a trypsin-like acidoproteinase. Similarly the enzyme hydrolysing carbobenzoxy-*l*-glutamyl-*l*-tyrosine may be called a pepsin-like acidoproteinase.
‡ Difference from hepatoma significant.

If one chooses dry or wet weight of liver as alternative reference points, the activity is nevertheless found to be significantly increased (Tables V and VI).

TABLE VI: COMPARISON OF ENZYMATIC ACTIVITIES, PER MGM. WET LIVER, OF HEPATOMAS WITH VARIOUS TYPES OF CONTROL LIVERS, USING 3 SUBSTRATES

Moles × 10^9 of substrate split per minute
pH 5.19-5.43

	BAA*	CGT	LG
Control	1.2† ± .40	1.8 ± .58	0.83 ± .38
Regenerating	1.0† ± .06		
Pre-hepatoma	1.4‡ ± .36		0.71 ± .36
Hepatoma	2.0 ± .38	1.9 ± .57	0.87 ± .18

* Substrate abbreviations and numbers of animals in each group are same as in Table V.
† Difference from hepatoma significant.
‡ Difference from hepatoma probably significant.

The results of enzymatic studies with other substrates are summarized in Tables V, VI, and VII. Each figure in these tables is the mean value of determinations on 5 or more separate livers.[5]

TABLE VII: COMPARISON OF ENZYMATIC ACTIVITIES, PER MGM. TOTAL NITROGEN, OF HEPATOMAS WITH VARIOUS TYPES OF CONTROL LIVERS,

Moles × 10^9 of substrate split per minute
C_4 pH5.19-5.43

	BAA*	CGT	LG
Control	46† ± 11	119‡ ± 42	31† ± 13
Regenerating	48† ± 2.1		
Fetal	56† ± 6.1		
Pre-hepatoma	69† ± 17		34‡ ± 14
Hepatoma	120 ± 17	210 ± 72	54 ± 13

* Abbreviations and numbers of animals in each group are same as in Table V.
† Difference from hepatoma significant.
‡ Difference from hepatoma probably significant.

DISCUSSION

It has previously been demonstrated by Bergmann, Fruton, and their co-workers (3, 4, 15) that specific peptide substrates may be used to characterize proteinases and peptidases more closely than is possible by means of protein substrates. Thus, the hydrolysis of benzoyl-*l*-argininamide by an extract of liver points to the presence of an enzyme designated by Bergmann as trypsinase. This liver trypsinase hydrolyzes the same substrate linkage as crystalline trypsin, but differs from the latter in its acid pH optimum and its dependence on an activa-

[5] The catheptic activity toward carbobenzoxyglycyl-*l* phenylalanine in a single hepatoma was compared with the activity in two normal control livers with the following results, expressed as $C_D \times 10^{-9}$: hepatoma 96, controls 46 and 62.

tor. Similarly, the liver enzyme which hydrolyzes carbobenzoxy-*l*-glutamyl-*l*-tryosine has substrate specificity requirements similar to those of pepsin. As stated previously, *dl*-leucylglycine is assumed to be split by an aminopeptidase. There is a possibility that the enzyme responsible for this hydrolysis is a dipeptidase, although doubt has been raised concerning the existence of an enzyme specifically restricted to the hydrolysis of dipeptides (9). Since the substrate carbobenzoxy-*l*-leucylglycylglycine is split, there is also the possibility that a carboxypeptidase may be present.

In comparing enzymatic activities in the liver it is useful to have more than one reference point, for the following reasons:

1. When the activity is related to protein nitrogen (or total nitrogen), an increase in activity may represent a loss of inert protein in the liver just as well as an absolute increase in the enzyme.

2. The water, lipid, and glycogen concentrations of the liver may undergo a change, thus influencing the enzymatic activity as related to the biological unit, the liver cell.

The question as to whether the changes observed between hepatomas and control livers are due to an increase in tissue concentration of a particular enzyme protein molecule, or to greater activation of an unchanged number of such protein molecules cannot be answered unequivocally. The term "activity" has therefore been used instead of "concentration" in order to encompass this uncertainty. It should be pointed out, however, that cysteine has been added to all test solutions in an effort to provide maximal activation of each enzyme molecule.

The problem of how best to prepare a series of individual livers for enzymatic comparison is an important one. The less refined the enzyme preparation is, the greater is the possibility that other proteins, enzymes, inhibitors and activators may influence the measured activity. On the other hand, the more purification procedures used the more difficult it becomes to obtain reproducible yields of purified enzyme from aliquots of the same starting material. The method herein used was worked out (27) as a compromise between these two difficulties. It is open to the objection that glycerol may provide incomplete extraction of enzymes from the tissue powders, but this situation should apply equally to hepatomas and controls.

It is conceivable that the enzymatic changes observed are related to an increase or change in normal connective tissue associated with the hepatoma rather than to the presence of the malignant cells, a possibility which requires further investigation.

Kishi, Fujiwara and Nakahara (16), using gelatin and casein as substrates, reported a decrease in catheptic activity in hepatomas as compared with using hemoglobin as a substrate, found an increase. normal livers; while Maver and her associates (19), In addition to the substrate differences, these investigations differed from the present one in other respects—animal strain, type of carcinogenic agent and extraction procedure—all of which make comparison of results difficult. It appears desirable to extend the present observations to include other types of tumors in order to find out whether increased catheptic activity toward benzoyl-*l*-argininamide is a specific property of this particular hepatoma or a more general property of tumors, and such a program is under way.

SUMMARY

A comparison of the activities of catheptic enzymes at pH 5 has been made in *p*-dimethylaminoazobenzene-induced hepatomas and in control livers. The activity of the enzyme responsible for the hydrolysis of benzoyl-*l*-argininamide was increased in the hepatoma. Changes occurring in the enzymes which hydrolyzed carbobenzoxy-*l*-glutamyl-*l*-tyrosine and *dl*-leucylglycine were of questionable significance.

An increase in total hepatic weight, water concentration, total nitrogen, and free cholesterol in the hepatomas as compared with control livers and pre-hepatomas, was also found.

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Studies in Carcinogenesis with Azo Compounds

III. The Action of (A) Four Azo Compounds in Wistar Rats Fed Restricted Diets; (B) N,N-Diethyl-*p*-Aminoazobenzene in Mice

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(A) FOUR AZO-COMPOUNDS IN WISTAR RATS FED RESTRICTED DIETS

The original experiments by Japanese workers who demonstrated the carcinogenic action of certain azo dyes were carried out using diets which were unbalanced in the same directions as the diet consumed by large sections of the Japanese population. Usually the basal diet consisted of 98 per cent polished or unpolished rice to which was added 2 per cent olive oil containing the azo compound under test. Green vegetables were usually fed as a supplement and also slices of carrot. The Japanese workers reported a considerable early mortality among rats fed such diets, but Rhoads and his collaborators who have used the same dietary formula reported (26) only a 3 per cent early mortality, presumably due to the superior nutritional value of the rice which was unpolished. Miller, Miner, Rusch and Baumann (20) regarded the rice-carrot type of diet as unsatisfactory because it usually led to early death of most of the rats and, moreover, was uncontrolled in regard to its constituents. They therefore experimented with two types of basal diet, one modelled on the rice-carrot diet of other workers and the other designed to be low in protein but otherwise adequate for growth. The "rice-carrot model" gave poor growth, especially when butter-yellow (*N,N*-dimethyl-*p*-aminoazobenzene, DAB) was added. The "low protein diet", however, gave reasonable growth, especially at the level of 12 per cent casein, and even when DAB was added early mortality was reduced to 25 per cent compared with over 50 per cent reported by various Japanese workers.

War-time conditions made it difficult to obtain supplies of glucose for animal experiments in Britain and, when work was begun to test the long-term action of azo-dyes in rats, we used a restricted diet, R. D. 1, in which the cerelose (glucose monohydrate) of the low protein diet of Miller and associates was replaced by boiled potatoes (small portions of skin and "eyes" were not always removed by the peeling machine).

RESTRICTED DIET, R.D.1.

	Per cent	Ratio of dry constituents	Low protein diet of Miller et al. (20)
Casein (Glaxo, unextracted)	12	12	12
Potatoes (peeled, boiled)	76	19	—
Glucose Monohydrate	—	—	77
Salt Mixture (Glaxo, L.D.6)	4	4	4
Dried Yeast (D.C.L.)	2	2	—
Arachis Oil	5	5	—
Cottonseed Oil	—	—	5
Cod Liver Oil	1	1	2
Vitab	—	— 4 drops/ rat/day	
	100		100

The manufacturers of the dried yeast claimed a riboflavin content of 50 mgm. per gm. of dry yeast. Owing to the high content of water in boiled potatoes the level of riboflavin in R. D. 1 was really about 200 mgm. per 100 gm. of dry diet. Assessed on the dry weight basis, the level of protein must also be regarded as double that given by Miller and associates, *i. e.* 24 per cent instead of 12 per cent. Thus a rat eating 10 gm. per day of the diet R. D. 1 was not short of either casein or riboflavin. In fact, control rats reared from weaning on R. D. 1 grew well, although not so well as rats on the usual basal diet, the rat cake of Thomson (27), used in this department.

Experiments involving the feeding of *p*-aminoazobenzene (AAB) or of DAB were carried out using the basal diet, R. D. 1. While these were in prog-

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ress, a supply of rice starch became available, and further experiments were begun using the diet R. D. 3 in which the carbohydrate component was rice starch:

Restricted Diet R.D.3

	Per cent
Casein (Glaxo, unextracted)	12
Rice Starch	76
Salt Mixture (Glaxo, L.D.6)	4
Dried Yeast (D.C.L.)	2
Arachis Oil	5
Cod Liver Oil	1
	100

This was a "dry" diet in the sense that no component contained any considerable quantity of water, and rats fed this diet could be regarded as on a low protein and, probably, low riboflavin diet. Certainly, the proportion of protein was not higher in our diet, R. D. 3, than in the low protein diet of Miller and associates (20).

Experiments Involving AAB and DAB

Wistar rats of both sexes, bred in this Department from rats originally obtained from Glaxo Ltd., were used for these experiments.

(a) *Controls on R. D. 1.*—Eight males and 8 females were given R. D. 1, without any dye, *ad libitum*. The average period of survival was 77 weeks; weight gains were good up to 40 weeks after which they usually ceased. Weight losses and death were usually due to bronchiectasis and consequent cachexia; bronchiectasis is not uncommon in our strain of rats, but was more frequent in rats fed R. D. 1 than in those fed rat cake.

The livers of these control rats showed no proliferative changes and seldom any degenerative changes. Four out of 11 rats showed necrosis of the convoluted tubules of the kidney; 1 female showed papillary hyperkeratosis of the stomach.

(*b*) *Rats fed R. D. 1 containing DAB.*—Eight male and 7 female rats were given the basal diet, R. D. 1. At first, DAB was added at the level of 0.06 per cent; later an attempt to feed it at 0.1 per cent was made, but weight losses followed and the level was reduced to the original. However, in the later stages it was found possible to feed these rats with R. D. 1 containing 0.07 per cent DAB. Feeding with the dye was continued up to 33 weeks; only 2 rats died before this period was completed and 1 of these animals had large liver tumors when killed at 29 weeks. The average length of life was 40 weeks but the latent period before liver tumors were palpable was about 33 weeks. Four females surviving 14 weeks or more after the dye was withdrawn had large liver tumors at autopsy, but little or no cirrhosis. Thus 7 males and 7 females surviving 200 days all developed liver tumors of various types, with metastases in one case, in 204 to 320 days. A 100 per cent incidence was therefore achieved in about 10 months. This is longer than the period recorded by Miller and collaborators (20) who reported 90 to 100 per cent incidence at 4 months on their low protein diet, and at first it was considered that the potato diet R. D. 1, retarded the formation of DAB hepatic tumors.

(c) *Rats fed R. D. 3 containing DAB.*—As mentioned above, it became possible to test the action of DAB given orally to our strain of albino rats in a diet, R. D. 3, in which the carbohydrate ration was rice starch, and which, therefore, approximated much more nearly to the low protein diet of Miller and associates.

The level of DAB in R. D. 3 was 0.07 per cent throughout. Rats in this group gained weight for a considerable period but failed to reach the same levels as did the rats given DAB in R. D. 1. Here also 2 rats were killed in the 29th week; the livers showed fatty degeneration and coarse bands of perilobular cirrhosis and in 1 male there were numerous cholangiectases. However, a female killed after 257 days had hepatomas, cholangiomas and bile-duct carcinoma. Of the remaining 9 rats, only 2 had tumors; a male killed at 306 days had hepatoma and adenocarcinoma of bile ducts, and a female killed at 372 days had liver cell carcinoma. Two others killed at 294 and 334 days respectively and 4 killed at 372 days showed fatty degeneration of the liver and cholangiectases; the last rat, also killed at 372 days, had no liver lesion except extensive fatty degeneration. Thus only 3 out of 12 rats surviving 200 days showed neoplasia of the liver, 8 others having cystic dilatation of the bile ducts without even hyperplasia. Therefore a 100 per cent incidence of liver tumors was not obtained after feeding DAB at a level of 0.07 per cent of the diet for 6 months. Even at 1 year the incidence was only 25 per cent. The diet R. D. 3, which was expected to favor carcinogenesis by DAB in view of the results of Miller and associates, was therefore much less favorable to hepatic tumor formation than was the earlier diet, R. D. 1. If our diet R. D. 3 and the low protein diet of Miller and associates are regarded as comparable, then our diet R. D. 1 is much more procarcinogenic, and the reason for the later appearance of liver tumors in

our rats must be due to the differences in the susceptibility of Wistar rats and Sprague-Dawley rats, or to some unrecognized differences in experimental technique. On the other hand, Sugiura and Rhoads (26) reported a 100 per cent incidence of liver tumors in Wistar rats fed DAB in the rice-carrot diet after 120 days. Tests with DAB in Sprague-Dawley rats maintained on our diet R. D. 1 would be of real interest here.

(*d*) *Rats fed R. D. 1 containing AAB.*—Sixteen male Wistar rats were put on the diet R. D. 1 to which was added 300 mgm. AAB per 100 gm. After 20 weeks the level of the dye was raised to 500 mgm. per 100 gm. diet and later (30 weeks) to 1.0 gm. per 100 gm. diet, but eventually (45 weeks) it had to be lowered to 200 mgm. per 100 gm. diet before weights could be maintained. The first rat died at 267 days, having consumed some 20 gm. of AAB. The spleen was greatly enlarged, and there was periportal cirrhosis of the liver, but no sign of hyperplastic changes in the bile ducts or liver cells. At 400 days a rat was killed, after about 26 gm. AAB had been consumed, and its liver showed areas of localized hepatoma containing large, foamy cells. Rats dying at 433, 471 and 519 days showed only degenerative changes, but the 2 dying at 525 and 527 days respectively had large tumors in the liver, which proved to be liver cell carcinomas, with secondary deposits in the mesentery in 1 rat. Bile duct proliferation and cholangiectasis were found but no cholangioma or adenocarcinoma of bile ducts. Of the nine survivors 4 (dying at 548, 620, 702 and 730 days) showed hepatomas, with or without bile duct proliferation, but no malignant growth. The other 5 rats died at 640, 673, 677, 683 and 841 days respectively; the last to die had cholangiectases, but no tumors were found in any of these animals. The last 3 rats had consumed about 35 to 36 gm. of AAB in 2 years' experimentation.

(*c*) *Rats fed R. D. 3 containing AAB.*—Eight male and 7 female Wistar rats were given the diet R. D. 3 containing 250 mgm. AAB per 100 gm. of diet. After 5 months, the dye level was reduced to 150 mgm., after 7 months to 100 mgm. and, finally, after 18 months, to 80 mgm. per 100 gm. of diet. In this way it was possible to keep 6 rats alive for 2 years, but the amounts consumed by all the rats were less than those consumed by those receiving R. D. 1 and living for the same period. The spleens were usually enlarged, especially those of the female rats and the proximal convoluted tubules of the kidneys were pigmented. Two female rats had ulceropapilloma of the forestomach, compared with 6 rats on R. D. 1 plus AAB, but these forestomach lesions are not considered to be directly due to the azo dye, as they resemble lesions found in this site in rats fed a great variety of diets and may be due to a deficiency of some labile component of crude casein (29). One rat died early at 129 days and showed no lesion of the liver. The remaining 14 rats died between 465 and 637 days except for the last 6 which were killed at 738 days. Apart from occasional fatty degeneration, the only abnormalty of these livers was the pigmentation of the Kupffer cells which were rendered unusually prominent thereby.

It seems unlikely that the somewhat lower concentration of AAB in the diet R. D. 3 can account for the complete absence of proliferative changes in the livers of the rats receiving this ration even after 2 years. It would seem that the diet R. D. 3 is not so favorable to liver-tumor formation as is the diet R. D. 1; the latter may prove to be of special value for some types of azo compound investigation.

(*f*) *Rats fed R. D. 3 containing an MAB derivative.*—The unsatisfactory results obtained by methylation of *p*-aminoazobenzene with methyl iodide according to the method of Berju (1) make it necessary to approach the synthesis of monomethyl aminoazobenzene (MAB) by some route yielding only the one product. Miller and Baumann (15) coupled benzene diazonium chloride with *N*-monomethylaniline and rearranged the diazo compound in the usual way. Another method is to block one position in the amino group of aniline and methylate the other using this derivative for the usual coupling procedure. The formaldehyde-bisulphite derivative is convenient for this purpose, as the formaldehyde-bisulphite group can be readily removed to yield the secondary amine. The ammonium salt of the formaldehyde-bisulphite compound of *N*-monomethyl-p-aminoazobenzene (AmFBMAB) was kindly prepared for us by Imperial Chemical Industries (Dyestuffs) Ltd. This compound is water-soluble but readily converted *in vitro* to MAB; it seemed of interest to feed rats with it and determine whether it is significantly converted *in vivo* to MAB.

Eight male and 8 female rats were given the basal diet R.D.3 containing 150 mgm. AmFBMAB per 100 gm. After 6 months the level was reduced to 100 mgm. per 100 gm. of diet and maintained at that figure until the end of the experiment, i.e. a further 18 months. Only 1 rat failed to survive a year, a female, dying at 336 days; the spleen in this animal was enlarged, the sinuses being distended with blood, while the liver showed a small degree of fatty degeneration and some periportal bile-duct prolifera-

tion. Males dying at 553, 575 and 577 days, and a female dying at 492 days showed no gross abnormality of the liver and this organ was not examined microscopicaly. Two other rats, a male dying at 473 days and a female killed at 587 days, showed no proliferative changes in the liver, that of the female rat showing widespread fatty degeneration. Of the other 9 rats, dying or killed at 451, 500, 515, 577, 692, 716, 739 and 739 days respectively, all showed cholangiectasis which, in 5 cases, caused clear superficial cysts in some liver lobes reminiscent of the cysts often seen in rats fed DAB. In the livers of those dying at 451 515 and 556 days respectively there were occasional areas of bile-duct proliferation; this was also seen in one of the rats killed at 739 days. A few nodules of hyperplastic liver cells were present in the livers of both the rats killed at 739 days. In one of the latter rats, the right ovary was replaced by a large spindle-cell sarcoma invading adipose tissue, but assumed to be of spontaneous origin The kidneys of all rats showed little damage, and spleens were also normal except in the one case mentioned above.

(g) *Rats fed R. D. 3 containing EAB.*—*N,N*-diethyl-*p*-aminoazobenzene (EAB) was synthesized according to the method of Gnehm and Bauer (6) and purified by passage in benzene solution through B.D.H.[1] alumina. Nine male and 10 female rats were given the diet R.D.3 containing 100 mgm. EAB per 100 gm. Six weeks later the level was reduced to 80 mgm. per 100 gm., after a further 6 weeks to 60 mgm. per 100 gm. and finally, after another month, to 50 mgm. per 100 gm. of diet this level was then maintained until the end of the experiment. Twelve rats survived more than 400 days; the longest periods were for a female killed at 612 days and a male killed at 635 days. In no animal did the liver show any significant change, either proliferative or degenerative. The spleens in 3 animals, and the kidneys in 4, showed fibrosis, while evidence of toxic damage to the kidneys was found in 5 rats of which 3 died before 210 days. There were no other remarkable lesions in any of the animals given this azo-dye.

DISCUSSION

The experiments recorded here in which Wister rats were fed DAB in two different types of restricted diet yielded rather surprising results. The extensive work of Baumann and his collaborators, using Sprague-Dawley rats, indicates that liver tumors are readily induced by DAB in their low protein diet. Moreover, Surgiura and Rhoads (26) found that Wistar rats all developed liver tumors by 120 days when fed DAB in the rice-carrot diet. Our diet R.D.3 differs from the low-protein diet of Miller and associates (20) only in replacing cottonseed oil by arachis oil and cerelose (glucose monohydrate) by rice starch. There is no reason to attribute the reduction in tumor incidence to differences between the oils used. The observation of Miller, Kline, Rusch, and Baumann (17) that hydrogenated coconut-oil gave considerable protection against liver carcinogenesis due to DAB as compared with corn oil is not relevant, as arachis oil has a chemical constitution even closer to that of corn oil than is that of cottonseed oil. Hydrogenated coconut oil differs radically in composition from the 5 oils known to favor liver tumor formation by DAB, namely olive, cottonseed, corn, and arachis oils and butterfat. Since diets containing no oil at all yielded a high incidence of tumors (18) the corn oil did not appear to be a positive co-carcinogen. On the other hand, addition of antioxidants or the feeding of diets containing the same amount of trilaurin as would be present in 5 per cent hydrogenated coconut oil failed to alter tumor incidences (18), and the definite anticarcinogenic action of hydrogenated coconut oil was still not explained. It seems possible that the action may be due in part to a nonsaponifiable constituent; hydrogenation alters steroids as well as fats and might yield a substance or substances not present in the favorable oils mentioned above. Kline and collaborators (13) now conclude that lauric acid is the principle in hydrogenated coconut oil which is responsible for the protective effect of diets containing the latter oil. Since there is no lauric acid in arachis oil there is no reason to expect arachis oil to be protective compared with cottonseed oil on this ground.

The replacement of cerelose by rice starch in our diet R.D.3 is more likely to have had some effect on tumor incidences. But as it has only just now become possible to carry out experiments with diets containing glucose as the source of carbohydrate, it is impossible to say yet whether this factor is significant or not. On the other hand, it may be entirely a question of differences in strain or even in sub-strain of rats. But the great increase in the liver tumor incidence found when starch was replaced by potatoes suggests that the carbohydrate component may be responsible. However, the large amount (75 per cent) of water in the boiled potatoes upsets the balance of this diet considerably and makes it very difficult to compare either with our R.D.3 with the diets used by Miller and associates (20).

The great value of the diet R.D.1 has been the fact that it enabled us to induce tumors of the

[1] British Drug Houses, Ltd.

liver in rats fed this diet containing large amounts of the free base, *p*-aminoazobenzene, for a long time. *p*-Aminoazobenzene has been regarded as noncarcinogenic since the early reports of Yoshida (28) and Sasaki and Yoshida (23) who obtained no liver tumors in white rats fed AAB in their rice-carrot diet up to 449 days. This finding has frequently been repeated in the literature. Recently, Miller and Baumann (16) carried out a comparative test on several azo dyes related to DAB, including AAB which they fed to Sprague-Dawley rats in their low-protein diet at the molar-equivalent level to that used for DAB itself. AAB was given for 8 months and the rats were allowed to live on the dye-free diet for a further 2 months, but even then the 9 survivors showed no gross lesions of the liver (microscopic examination was not reported). Hence AAB is clearly a very much weaker carcinogen for rat liver than is DAB.

The observation of Kensler, Dexter, and Rhoads (7) that the theoretical split-product of AAB, namely *p*-phenylenediamine, has, under suitable condition, a considerable inhibitory action on a diphosphopyridine nucleotide system but only about one-third that of the theoretical split product from DAB, namely *N,N*-dimethyl-*p*-phenylenediamine, led them to postulate that azo compounds yielding split-products with enzyme inhibitory powers of less than a certain threshold would be noncarcinogenic. However it would be just as logical to argue that any azo compound yielding a split-product which was inhibitory towards enzyme systems will be carcinogenic if administered in a suitable way. In the latter case, *p*-aminoazobenzene should be carcinogenic for rat liver, albeit a very weak carcinogen. In point of fact, hepatoma formation was caused in 7 out of 16 rats given AAB orally up to 2 years in our diet R.D.1. In only 2 cases were the tumors malignant and carcinoma of bile ducts was never seen. The action of AAB seems, therefore to be predominately on parenchyma liver cells, whereas that of DAB is primarily on bile ducts (21). A preliminary note on these AAB tumors has already been published (9) and, in collaboration with Dr. P. R. Peacock, a study of the comparative histology of liver lesions due to AAB or DAB together with full details of the relevant experiments is shortly to be published (12).

In a recent paper, Miller and Baumann (14) have criticized the results reported in the preliminary communication. They suggest that contamination of the AAB used with small amounts of DAB, or the *in vivo* methylation of part of the AAB, could account for the low incidence of liver tumors obtained To these points it may be replied that (a) the method of synthesis of AAB (by intramolecular rearrangement of diazoaminobenzene) precludes the possibility of the presence of DAB and it is believed that precautions to prevent subsequent contamination were adequate; (b) if the tumors were due to DAB, however introduced, one would have expected to see some cases of bile duct tumors which are characteristic in our experience, as in the experience of Orr (21), whereas in fact the tumors were exclusively hepatomas.

Miller and Baumann (16), and Giese, Miller, and Baumann (5), have shown that *N*-monomethyl-*p*-aminoazobenzene (MAB) is as active as the fully methylated compound, DAB, in inducing liver tumors in Sprague-Dawley rats given the dyes orally. This finding was confirmed in a concurrent but independent investigation by Sugiura, Halter, Kensler and Rhoads (25) who used Sherman rats, but did not state how they prepared their specimen of MAB which Miller and Baumann (15) had to prepare by a new route, the previously recorded preparation by Berju (1) giving a product of quite different melting point. The present author was also unable to prepare MAB by Berju's method.

According to the split-product hypothesis, MAB, which yields the theoretical split-product, *N*-monoethyl-*p*-phenylenediamine, having an enzyme-inhibitory power about half-way between those of *p*-phenylenediamine and *N,N*-dimethyl-*p*-phenylenediamine, should have a carcinogenic activity about half that of DAB. It was found to have the same activity as DAB, but this discrepancy has been explained by the observations of Miller, Miller and Baumann (19) who found that MAB given orally to Sprague-Dawley rats was both demethylated to AAB and further methylated to DAB. DAB itself is demethylated successively to MAB and to AAB. Hence oral administration to these rats either of DAB or of MAB yields both compounds *in vivo*, and the carcinogenic outcome will be the same in either case. The complete inability of the rat liver to methylate AAB, which these authors also discovered, is a curious fact when one considers the ability of the rat to use labile methyl groups to transform aminoethanol into choline *i.e.* a free amine to a quaternary amonium compound (24).

The failure of the ammonium formaldehyde-bisulphite derivative of MAB to induce lesions other than cholangiectasis in the liver of rats fed this compound suggests simply that the rat is unable to remove the formaldehyde-bisulphite group *in vivo* to any significant extent. The water solubility of this compound will, of course, have an effect on its course through the organism; metabolic studies have

not been made. The pathogenesis of the cholangiectasis is not yet clear as it is not always accompanied by cirrhosis.

The higher homologue of DAB namely *N,N*-dimethyl-*p*-aminoazobenzene (EAB) was found by Sugiura and collaborators (25) to be quite inactive when fed to Sherman rats in the "rice-carrot" diet up to 511 days. Our results indicate that Wistar rats do not develop neoplastic lesions of the liver when fed EAB in our diet R.D.3 for up to 635 days. There remains the possibility that EAB, given orally in our potato diet, R.D.1, which proved so effective in the case of AAB, might yield tumors of the liver in our rats, but this could only be ascertained by a long-term experiment.

The "Split-product" hypothesis.—If EAB is completely inactive, this fact will have an important bearing on the "split-product" hypothesis of Kensler and associates (7). Miller, Miller, and Baumann (19) showed that DAB was demethylated by the rat liver to yield MAB and finally AAB; the ratio of these 3 compounds present in the liver of rats fed either MAB or DAB was DAB, 2: MAB, 1: AAB, 10. Thus the greater part of any DAB (or MAB) given to the rat cannot yield the *N,N*-dimethyl-*p*-phenylenediamine which Kensler and associates postulated as the active derivative from DAB. In the case of *N*-ethyl compounds, the evidence available suggests that de-ethylation does not occur (2). Hence reductive fission would yield *N,N*-diethyl-*p*-phenylenediamine. Recently, Elson and Hoch-Ligeti (3) have published the results of their investigations into the inhibitory action of various diamines towards urease and succinoxidase. They have examined the action of, among a number of amines, *p*-phenylenediamine and its *N,N*-dimethyl and *N,N*- diethyl homologues on urease activity, and found that all 3 will cause 100 per cent inhibition if the amine solution is allowed to stand long enough for oxidation to reach a maximum. With freshly prepared solutions, the percentage inhibitions of the 3 amines in question was 36, 60 and 30. After standing 1 day, the dimethyl diamine had 100 per cent inhibition; the diethyl diamine reached this level of activity after standing 2 days, but the free diamine only after 4 days. If carcinogenicity runs parallel with enzyme-inhibitory power, the order of carcinogenicities should be DAB > EAB > AAB. The evidence available shows the order to be DAB > AAB > EAB. It would have been of some advantage to know the relative toxicity towards urease of 2,5-diaminotoluene which Kensler, Dexter and Rhoads (7) found to inhibit a DPN system to a rather greater extent than did *p*-phenylenediamine.

As AAB and 4′-amino-2,3′-azotoluene are both capable of inducing liver tumors in rats fed either compound, the presence of *N*-methyl groups is clearly not essential. But the absence of activity in the higher homologues of DAB (25) indicates that lability of substituents at the amino group is very important, and substitution with methyl groups seems the most effective way of enhancing the carcinogenic activity of AAB. The variety of responses elicited by feeding with *o′*, *m′*, or *p′*-methyl derivatives of DAB (16) may be related to the relative ease with which demethylation occurs in these compounds.

The "benzidine-rearrangement" metabolism of azo-compounds, demonstrated in the rat by Elson and Warren (4), may provide a hypothesis relating carcinogenicity to metabolism, which is not subject to the criticisms weighing so heavily against the "split-product" hypothesis. So far the rearrangement product from DAB, namely 2,4′-diamino-5-dimethylaminodiphenyl, has been shown to be formed *in vivo* from DAB and to be toxic towards urease and succinoxidase systems. Until it has been shown that other carcinogenic azo-amines yield similar rearrangement products and, if so, that these products are toxic toward enzyme systems, the "benzidine-rearrangement" hypothesis cannot be assessed. In any event, the greater activity of DAB compared with 4′ amino-2,3′-azotoluene in rats when the latter is more active than the former in mice remains to be explained.

(B) *N,N*-DIETHYL-*p*-AMINOAZOBENZENE IN MICE

The literature on the action of azo compounds in mice has been reviewed recently (11). While DAB has been shown to cause tumors in mice, both at the site of injection and in the liver no tumors were seen in mice injected subcutaneously with AAB. Tests with EAB in mice have not been reported.

Two groups of mice of both sexes were selected for carcinogenicity tests with EAB; one group consisted of ordinary stock mice of mixed colors, the other of mice of the Cba strain, which had responded with liver tumors following the subcutaneous injection of DAB in arachis oil (10). The solvent used was again arachis oil in which EAB is readily soluble; a 5 per cent solution does not crystallize out at room temperatures. The basal diet was rat cake; experiments with our restricted diet, R.D.1 were not attempted in this series.

(*i*) *Experiments with stock mice.*—Altogether, 36 male and 34 female mice were used; 32 males and 31 females were examined post-mortem. The first in-

jection into 28 males and 22 females consisted of 0.25 ml. of a 3 per cent solution of EAB, comparable with that used in the tests with DAB; 11 males and 13 females died within the first week. The remainder were subsequently injected with a 1 per cent solution; an additional 10 males and 14 females received injections of the 1 per cent solution only. Mice surviving 423 days after the beginning of the experiment received 10 injections of 0.25 ml., and 4 injections of 0.5 ml. of the 1 per cent solution. Thus the maximum total dose was 55 mgm. per mouse; only 4 mice received this amount of EAB. Injections in alternate flanks. Mice surviving the last injection were allowed to live as long as possible.

Tumors were not found at the site of injection. Only 2 mice, a male surviving 252 days (35 mgm. EAB) and another male surviving 451 days (55 mgm. EAB), showed regenerative hyperplasia of the liver parenchyma and bile duct proliferation or hypertrophy was not seen.

Ten males, surviving from 1 to 462 days showed no abnormality of the liver in section; all livers of females (13 examined in section) showed lesions. Focal necrosis and/or fatty degeneration of the liver were seen in 7 of 11 dying up to 7 days, in 13 out of 17 dying between 7 and 268 days, but in none out of 7 mice dying between 405 and 622 days. Hyaline (waxy) degeneration was seen in 3 livers from the medium—and long-lived groups.

While 8 out of 32 mice had normal kidneys (in section), 7 out of 11 dying before 7 days had considerable necrosis of the convoluted tubules and in 5 of these the glomeruli were also degenerate. Cystic dilatation of the tubules, usually with debris present, was found in 6 out of 12 surviving 30 to 622 days. Hyaline waxy degeneration was found in 2 mice only.

Only 1 in 27 spleens examined in section was normal. Five spleens showed hyaline degeneration of the pulp around the malpighian bodies, mainly from mice dying early. Twelve spleens contained megakaryocytes, often in considerable numbers, again mainly in those mice in the short—and medium-lived groups, but also in 1 female dying at 432 days.

One male, dying at 405 days, had an adenoma of the lung, considered to be spontaneous. No other abnormalities were seen in any of these mice.

(*ii*) *Experiments with Cba mice.*—25 males and 39 females were used. Seven males and 9 females were injected initially with 0.25 ml. of 3 per cent EAB in arachis oil; none survived a week. Eighteen males and 26 females were then injected with 0.25 ml. of 1 per cent solution; 6 males and 16 females died within a week. The survivors, and another 4 female mice, were injected subsequently with a 0.5 per cent solution; but after 220 days from the first injection of this group a 1 per cent solution was used again, and 0.5 ml. injected per mouse. Injections in this group also were subcutaneous, in alternate flanks, at not less than 3 weeks' intervals, but the maximum amount of EAB injected per mouse was only 42.5 mgm.

No tumors were found at the sites of injection or in any organ. Eight out of 14 livers examined in section were normal, mainly in the medium-lived mice. Three out of 4 livers of short-lived mice showed focal necrosis, and this lesion was found in the livers of both long-lived mice. No regenerative hyperplasia was seen in the livers of any mice in this group. Similarly, 3 out of 4 short-lived mice had necrosis of convoluted tubules of the kidney, 3 out of 9 medium- and long-lived having normal kidneys and 2 out of 7 medium-lived having cystic dilatation of the convoluted tubules. Of 11 spleens examined in section, none was normal; megakaryocytes were seen in only 2, but 4 showed hyaline degeneration of the pulp around the malpighian bodies.

Comment on mouse experiments.—The statement by Kinosita (8) that "diethylaminoazobenzene is less poisonous" than *N,N*-dimethyl-*p*-aminoazobenzene (DAB) is not borne out by the experiments described here, either in rats or mice. Injections of the same *N,N*-diethyl-*p*-aminoazobenzene amount of (EAB) as of DAB into stock mice were much more toxic, and Cba mice failed to survive a week after such injections of EAB. As has been stated in an earlier section of this paper, EAB could not be fed to rats at as high a level as could DAB. Hence it would appear that EAB is really more toxic than is DAB toward both rats and mice.

This toxicity of EAB made it impossible to administer such large quantities of EAB to mice as were administered of DAB (10). However, a considerable number of mice were used; stock mice survived up to 622 days, and Cba to 541 days, without even hepatoma being found on gross or microscopic examination. The conclusion may therefore reasonably be drawn that EAB is not carcinogenic for the subcutaneous tissues or the liver of stock or Cba mice.

An interesting feature of this experiment with EAB in mice has been the histological picture evoked in a considerable number of spleens. The typical picture of hyaline degeneration around the malpighian bodies, reported by Parsons (22) for mice treated with pentose nucleotides or bearing grafts of a methylcholanthrene-induced sarcoma, was seen

in 5 out of 27 spleens examined from stock mice, and in 4 out of 9 from Cba mice, a total of 9 cases in 36 mice. Similar lesions have been seen in mice after a number of experimental procedures and these are summarized in Table I.

TABLE I: INCIDENCE OF HYALINE (WAXY) DEGENERATION AROUND MALPIGHIAN BODIES IN SPLEENS OF MICE RECEIVING TOXIC SUBSTANCES

Substance	Strain	Route	Ratio spleens showing lesion to total spleens examined
1. Benzpyrene } Methylcholanthrene }	Stock	Oral	0/19
2. 3.4.5.6-Dibenzcarbazole	"	Painted Injected	1/7 0/11
3. N-Methyl-3,4,5,6-dibenzcarbazole	"	Painted Injected	4/10 1/12
4. Extract of saponified liver (primary carcinoma)	"	Subcutaneous	1/12
5. Extracts of saponified bile:	"	"	1/1
(a) Rabbit	"	"	1/3
(b) Ox	"	"	0/27
(c) Human			
6. Diazoaminobenzene	"	Oral Painted Subcutaneou	2/8 2/12 3/14
7. Azo-dyes:			
(a) p-Aminoazobenzene	"	Subcutaneous	0/19
(b) N,N-dimethyl-p-aminoazobenzene	Stock, Cba, C57 Black	"	0/25
(c) N,N,-diethyl-p-aminoazobenzene	Stock, Cba.	"	9/36
(d) 4'Amino-2:3'azotoluene	Stock, Cba, C57 Black	"	0/40
(e) 2'Amino-4:5'azotoluene	Stock	"	0/7

It will also be seen from Table I that such splenic lesions were *not* seen in mice injected with 4 azo dyes (10). Thus the action of EAB appears to differ sharply, in this respect, from that of AAB, DAB or the aminoazotoluenes employed. It is unfortunate that these hyaline zones have never stained metachromatically for amyloid in our hands whereas the lesions obtained by Parsons in Cba mice stained with methyl violet, gentian violet and Congo red; probably differences in histological technic are responsible as our hematoxylin-eosin sections have all the appearances of amyloid infiltration. However, the significant point seems to be that the most toxic of the 5 azo dyes tested in mice in this Department has induced hyaline degeneration in a quarter of the spleens examined, whereas the 4 dyes exhibiting a lower toxicity induced no such lesion.

SUMMARY

1. *p*-Aminoazobenzene and *N,N*-dimethyl-*p*-aminoazobenzene have been given orally to Wistar rats in one or other of two restricted diets.

2. The incidence of liver tumors was reduced when boiled potatoes (in the first restricted diet, R.D.1) were replaced by starch (in the second restricted diet, R.D.3). Liver tumors due to *p*-aminoazobenzene were not obtained on the second restricted diet, R. D. 3.

3. The ammonium salt of the formaldehyde-bisulphite derivative of *p*-monomethylaminoazobenzene (water-soluble), given in the diet R.D.3, caused only cholangiectasis, with slight bile-duct proliferation and regenerative hyperplasia of liver cells, after 2 years' administration.

4. No significant liver lesions followed the oral administration in the diet R.D.3, of *N,N*-diethyl-*p*-aminoazobenzene up to 635 days.

5. *N,N*-diethyl-*p*-aminoazobenzene was also injected subcutaneously in arachis oil solution into stock mice of the Cba strain up to 622 and 541 days respectively; no neoplastic changes were seen in the livers of any of these mice, nor tumors at the site of injection.

6. *N,N*-diethyl-*p*-aminoazobenzene proved to be more toxic than the *N,N*-dimethyl derivative (butter-yellow), both for the rat and for stock and Cba mice, and was therefore administered at a lower level. The diethyl compound also induced hyaline (waxy) degeneration of the spleen in a proportion of both stock and Cba mice.

7. The data presented are discussed with reference to current theories of azo dye carcinogenesis.

ACKNOWLEDGMENT

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Tumor of the Lung in Rats Following Injections of Urethane (Ethyl Carbamate)*

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Following our 1939 paper (1) on treatment of transplanted rat carcinoma, in which it was shown that x-rays were notably more lethal to growing cancers in colchicine-treated rats than to similar cancers in untreated controls, we planned to extend the experiments but, because of war conditions, were unable to secure colchicine. A supply of urethane (ethyl carbamate, $NH_2\ COOC_2H_5$) was available, however, and since this drug was supposed to have effects on mitosis similar to those of colchicine we proceeded with it. It not only had a delaying effect on mitosis for some 12 to 16 hours, but what is more important, we found from later experiments, of which this is a report, that it induced tumors of the lung in a majority of our experimental animals. Since urethane is sometimes used as a human anesthetic, particularly for children, it seemed important to explore this lead further.

Nettleship and Henshaw (6) have reported induction of pulmonary tumors in mice with urethane. An inspection of this paper and the later ones of Henshaw and Meyer (3, 4) convinced us that we were obtaining in rats the same effects they got in mice; namely, the induction of multiple pulmonary tumors of adenomatous type. After completion of our present manuscript a study of Jaffé (5) has appeared in which he reports carcinogenic action of ethyl urethane on rats. He secured pulmonary adenomas both by injection and by feeding. Some of his animals also developed tumors of the liver. In our rats, a highly inbred stock, we found no involvement of the liver.

MATERIALS AND METHODS

Because the rat is far less likely than the mouse to develop cancers spontaneously, and particularly because we had a strain of rats selected for its 25 years of immunity to cancer implants (Flexner-Jobling carcinoma), it seemed worth while to continue our experiments with urethane. After some experimentation with intraperitoneal dosage, our treatments were standardized to injections of 1 cc. of a 10 per cent aqueous solution of urethane per 100 gm. of body-weight of the rat being treated, all of which were young adults. This was an anesthetizing dose which left the animal inert for several hours. The rats were kept in a room temperature of about 72° F.

Most of the animals received from 3 to 5 injections in all, administered at weekly intervals, although in certain cases only 1 or 2 injections were given. The practice was in each lot of animals, after the first injection, to leave certain ones uninjected each time so that at the end of 5 weeks the group would include individuals having had 1, 2, 3, 4, and 5 injections respectively. After the fifth, no further injections were given and about 11 months later all survivors were killed and autopsies made. Animals that died in the meantime were also dissected and carefully inspected.

The animals received the same diet as that fed regularly to our large rat colony; namely, Wayne Dog Food Blox, which analyzes into:

Protein	(Min.)	22.0%
Fat	(Min.)	4.0%
Carbohydrates (nitrogen free extract)	(Min.)	40.0%
Fiber	(Max.)	5.0%

In addition, a bit of fresh lettuce was given each day.

RESULTS

In all, 3 series of experiments were made on rats In the first set of observations 8 of 11 animals developed lung tumors. In our second experiment

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28 animals (14 males and 14 females) were used—all from a strain selectively bred for many years for immunity to carcinoma transplants. Twenty-three animals developed tumors in the lung. In the third series of unselected though highly inbred rats, 35 of 52 animals revealed pulmonary tumors upon autopsy.

Some 8 to 10 months after the first injection of urethane the treated animals commonly showed evidence of illness, lost weight and died within a month or two, if not killed for study. Inspection usually showed scattered tumor nodules beneath the pleura of the lungs, ranging in size from barely perceptible dots up to 4 or 5 mm. in diameter (Fig. 1), with sections showing occasional nodules more deeply embedded in the lung parenchyma. Males and females were equally affected. Although careful inspection was made of the other internal organs, notably the liver, no tumors were ever found apart from the lungs.

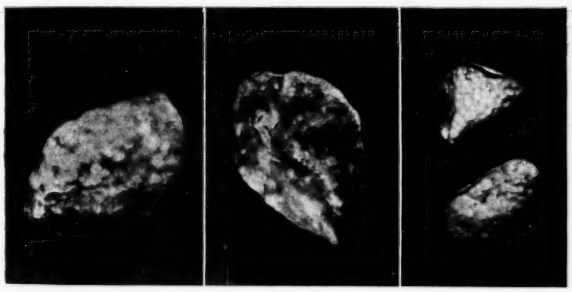

FIG. 1.—Rats' lung showing adenomatous nodules (whitish areas.)

Microscopic inspection showed many of the tumor cells making their first appearance as gland-like cells associated with the bronchioles (Fig. 2). As early as 8 to 10 weeks after the first treatment, small cellular foci which seemed to precede the tumors were detectable. As each such focal region increased in size the air sacs with which it was in contact became obliterated and replaced by adenomatous tissue. The simultaneous appearance of the nodules indicates that the neoplastic growth does not originate in a single cell but that a number of cells must have transformed into a similar form of neoplasm about the same time.

The general structure of the tumors, stained with hematoxylin and eosin and seen at different magnifications, is shown in Figs. 2 to 5. In Fig. 2, under low magnification (×48), the tumor tissue is plainly visible at the left, the much looser normal lung tissue, at the right. In Fig. 3, at a magnification of × 192, and in Fig. 4 at a magnification of ×516, the structure of the tumor itself is shown in greater detail. In Fig. 5, the characteristic appearance of the nuclei of the irregularly arranged tumor cells is seen at a magnification of × 1164. All of the tumors studied were of the same histological type.

That a single dose is sufficient to cause tumor formation is shown by the fact that 3 animals receiving but 1 injection each, developed tumors although not as plentifully as rats receiving multiple injections. Our records show, however, that following only 2 injections, as many as 37 tumors could be counted on the lung surfaces after the death of the animal.

In not a single one of our 50 control rats did tumor of the lung appear, although 1 had cancer of the liver. The several suspicious-looking nodules observed in 5 of the controls proved under microscopic examination to be infections. In several of the treated animals similar non-tumorous centers of bacterial infection were also observed.

Unlike colchicine, which delays mitosis in metaphase for from 15 to 25 hours, although permitting continuance of chromosome divisions, urethane seems to do away with practically all mitotic activity. Inspection of the graphs reproduced in Fig. 6 shows how nearly mitotic activities were brought to a standstill in corneal cells. They summarize the total counts of mitotic activity, made from separate studies of each microscopic field for prophase, metaphase, anaphase and telophase figures.

These graphs are based on sections in which mitotic figures of all phases were counted in every fifth section, with 36 sections counted for each cornea. Forty-two rats (21 males, 21 females) were injected at 9:30 A.M., after which 6 injected and 6 control animals were killed at the following hours: 10 A.M., 2 P.M., 6 P.M., 10 P.M., 2 A.M., 6 A.M., nad 10 A.M. The lower graph shown in Fig. 6 is for the 21 injected female rats and their controls. That for the males was not essentially different. The continuous line in the graphs represents the urethane-treated animals, the dotted line, the controls. The horizontal readings indicate the time of day the animals were killed, the upright column shows the average number of division figures per section in 108 sections (3 × 36) of the 3 female rats killed at any one time. For purposes of comparison a set of counts was made on 28 mice, although the counts for each phase were made on 2 instead of 3 individuals.

It will be noted that the controls show the fairly regular diurnal rhythm of mitotic activity commonly seen in mice and rats. Urethane, while not obliterating it, depresses it materially. For from a short time after 9:30 A.M. until about 2 A.M., a

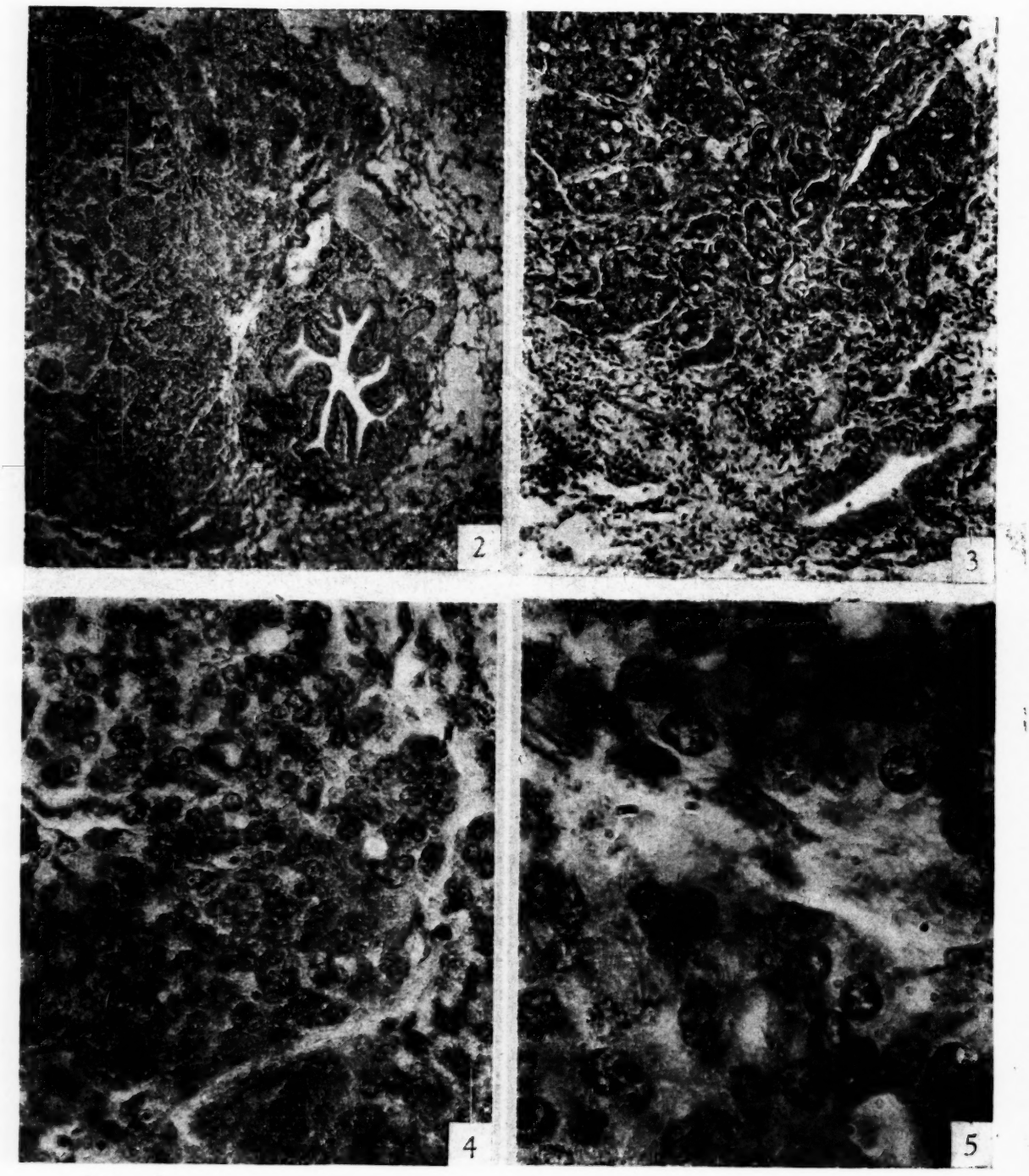

FIG. 2.—Tumor tissue at left, resembling glandular structures associated with a bronchiole. Mag. × 48.

FIG. 3.—Tumor structure is shown in detail. Mag. × 192.

FIG. 4.—Same as Fig. 3. Mag. × 516.

FIG. 5.—Characteristic appearance of nuclei of the irregularly arranged tumor cells. Mag. × 1164.

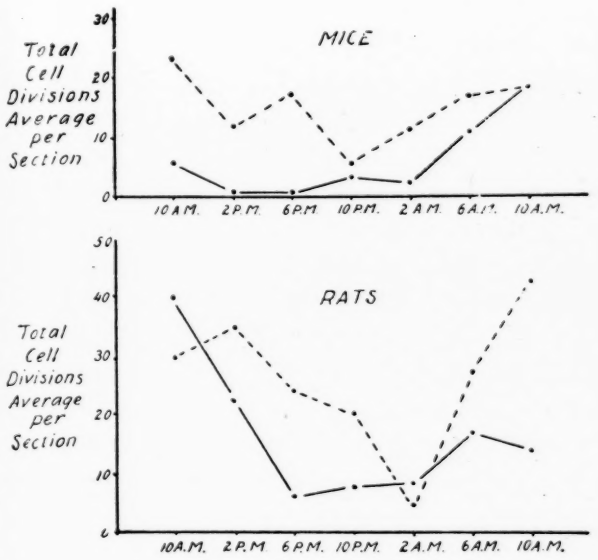

FIG. 6.—Graphs of effect of urethane on mitotic activity of cells of cornea in rats. They summarize total counts of mitotic activity, made from separate studies of each microscopic field for prophase, metaphase, anaphase and telophase figures.

period of about 16 hours, the mitotic count, including all stages, is low or lacking, after which it slowly mounts again. There was little or no variation from rat to rat. A more detailed description of the effects of urethane on mitosis will be found in our report published elsewhere (2).

It is evident from this study that, as affecting mitosis, urethane does not operate in the manner of colchicine by arresting mitosis in metaphase and holding it there, but does away almost entirely with all stages of it for a time. Its effects resemble more closely those of such anesthetics as ether or cocaine, or such mydriatics as ephedrin, which apparently prevent cells from entering upon mitosis. After injections of urethane the several phases of mitosis seem to disappear with equal rapidity, hence more than mere prevention of its inception seems involved. What part, if any, this suppression of mitosis plays in the imitation of pulmonary tumor formation, remains unanswered. It may be merely an accompanying response to the causative agent. It is obvious, however, that the drug has cytological effects on tissues other than those of the lungs. Further studies on the cytological aspects of the problem are being continued.

SUMMARY

Intraperitoneal injections of urethane (1 cc. of a 10 per cent aqueous solution, per 100 gm. of body-weight) induced the formation of multiple pulmonary adenomatous tumors in the majority of 91 rats so treated, including one lot of 28 which had been selectively bred for many years for immunity to carcinoma transplants. Cytological studies of other cells of the body, such as corneal cells in which division figures are easy to observe, showed that the drug noticeably reduced all stages of mitosis for some 12 to 16 hours, thus demonstrating that it has direct or indirect cytological effects not only on lung tissue but on other tissues as well, even though no tumors were found elsewhere. The study indicates also that a number of pulmonary cells underwent similar abnormal change at about the same time—that the neoplastic growth did not originate in a single cell.

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Anlage Tumors of the Salivary Glands

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Recently, Hellwig (11) reviewed the principal theories of the cellular origin of so-called salivary gland tumors. He concluded that they are derived from misplaced elements of the notochord. A group of salivary gland tumors was segregated by Stewart, Foote and Becker (18) and designated as "muco-epidermoid" tumors. For this type they implicated the salivary gland ducts as the anatomic site of origin. Another group was called "adenomyoepithelioma of the palatal mucous glands," by Bauer and Fox (3). These were believed to arise from the cells lining the intercalated ducts of the palatal mucus glands.

Our inquiry into the structure of the so-called salivary gland tumors leads to the conclusion that they are derived from embryonal rests of the ectoderm. The variations in cellular patterns suggest that the anlage separated at a stage when the cells as yet had not acquired their dominant characteristics as ectodermal or mesodermal cells. This explains their capacity to form ectodermal and mesodermal structures.

In this study only those growths believed to be benign and presenting the usual microscopic structure of salivary gland tumors were included. The records and specimens of 38 patients with salivary gland tumors observed during the past 15 years at the University of Oklahoma Hospitals were studied. The age, sex, and race of the patients, sites of the growths and their duration were also briefly considered (Table I).

Age, sex, and race.—Three of the patients were in the second, 4 in the third, 5 in the fourth, 13 in the fifth, 7 in the sixth, 5 in the seventh and 1 in the eighth decade of life. The youngest patient was 14 and the oldest 72 years of age. Nineteen patients were male and 19 were female. Four of the patients were negro (2 male and 2 female).

Site and duration.—In 29 patients, the growths occurred in or about the parotid glands (18 on the left and 11 on the right); in 6 patients, in or about the submandibular glands, (3 on the left and 3 on the right); and in 3 patients in the maxilla (right antrum, left hard palate and hard palate, respectively). The time interval between the first appearance of the growth and its removal varied. The growths about the parotid and submandibular glands were

TABLE I

Patient's number		Age	Sex and race	Side	Duration, years	Size cm.
PAROTID GLANDS						
P-1	1264-41	14	F W	L	3	4x4
P-2	1107-39	26	M N	R	14	7x8x5
P-3	1500-42	29	F W	R	8	3x3x2
P-4	178-37	30	F W	L	6	8x8
P-5	1715-37	31	F W	L	8	5x5
P-6	1250-45	35	MW	R	19	5x5
P-7	859-45	38	F W	L	20	3x3
P-8	790-32	42	MW	L	9 mo.	2x1x1
P-9	785-38	42	F W	R	22	5x5x4
P-10	959-38	42	F N	L	7	3x3
P-11	127-44	42	MW	R	4	6x5x3
P-12	151-42	43	F W	R	5	3x3x2
P-13	1235-41	44	MW	L	20	8x6
P-14	1690-37	47	MW	R	10	8x8
P-15	5-39	47	MW	L	27	5x5
P-16	462-46	48	MW	R	8	1x1
P-17	1083-42	50	F W	R	18	5x6
P-18	1411-36	52	MW	L	17	3x3
P-19	480-40	54	F W	L	11	20x15x8
	1302-40					5x5
P-20	2195-40	55	F W	L	4	2x2
P-21	883-43	56	F W	L	6	4x3
P-22	1264-46	56	F W	L	7	4x3x2
P-23	318-46	58	F N	L	2	3x3x2
P-24	1333-38	64	MW	R	5	5x3x2
P-25	233-45	64	F W	L	3	3x3
P-26	205-41	67	F W	L	16	8x8
P-27	1302-38	68	F W	R	1	3x2
P-28	713-37	70	MW	L	44	5x5
P-29	1158-38	72	MW	L	8	4x3x3
SUBMANDIBULAR GLANDS						
S-1	1891-41	28	MW	R	4	5x4
S-2	1399-37	32	MW	L	9	3x2x2
S-3	1032-38	42	F W	L	12	7x5
S-4	1060-37	43	F W	L	30	3x2
S-5	1841-41	50	F W	R	4	3x2
S-6	265-44	55	MW	R	3 mo.	5x4x2
MAXILLA						
M-1	144-45	15	MW	Palate	3 mo.	2x2x1
M-2	704-45	17	M N	L palate	2 years	7x5x2
M-3	1577-43	34	MW	R antrum	1 mo.	5x1x1

removed within 1 year of their appearance in 3 patients between 1 and 5 years in 9, between 5 and 10 years in 10, between 10 and 20 years in 9, and between 20 and 44 years in 4 of the patients. The growths in the maxilla, however, were removed much earlier, *i. e.*, 1 month, 3 months and 2 years, respectively, after they were first noticed.

Structure.—The growths ranged between 1 and 20 cm. in diameter, but most of them were not over 6 cm. They were fairly firm, globular and frequently lobulated. Their cut surfaces varied and presented no uniform pattern. Occasional septa subdivided the gray white nodular fields. The growths were usually surrounded by a more or less delicate capsule. However, occasionally no definite line of cleavage separated the growth from the surrounding structures. There was no fundamental difference in the gross appearance of the growths arising in the parotid regions and those situated elsewhere.

The microscopic appearance of the growths arising at any of the sites was closely similar, if not identical. Neoplastic epithelial elements were enclosed within neoplastic connective tissue, both in varying degrees of differentiation. Frequently, an apparent transformation of epithelial elements into connective tissue elements could be observed.

In all growths, sheets of epithelial cells forming no particular patterns or structures were seen in nests or strands connected with one another (Fig. 1). Elsewhere, a stream-like or concentric arrangement of the epithelial cells was clearly discernible with intercellular bridges and occasional keratinized round masses forming epithelial pearls (Fig. 2). Lumina surrounded by epithelial cells resembling the structure of ducts of sweat glands were occasionally seen. Other lumina, small or large, were lined by cuboidal or columnar cells with several rows of cell nuclei. In the larger cell nests pseudolumina, empty or containing a pink amorphous material, were seen singly or in varying numbers. A degree of differentiation to acinar tubular structures lined by cuboidal or columnar cells, producing a mucinous or serous secretion, and arranged in a pattern forming glandular structures was only occasionally observed (Fig. 3).

The neoplastic connective tissue elements forming the stroma, like the epithelial elements, varied in the degree of their differentiation. There were fields of embryonal connective tissue composed of cells with stellate processes fading into a barely stainable ground substance (Fig. 4). There were other fields of young connective tissue producing collagen. Fields of adult connective tissue, of embryonal young or adult hyaline cartilage, and of osteoid or osseous tissue were also seen. Quite frequently a barely perceptible transformation of the pink-stained ground substance into cartilage could be observed (Figs. 1 and 2). Elsewhere epithelial cells were seen scattered in nests, or spread apart into single file and transformed into cells of the cartilage (Fig. 5). A mucin-like fibrillar ground substance with scattered groups of epithelial cells was observed in some of the growths, fading gradually into young hyaline cartilage (Fig. 6). In some instances, there were scattered groups of adipose tissue cells (Fig. 7). In an occasional growth, groups of round or oval empty spaces were seen lined by flat cells resembling endothelial cells. The lumina appeared in groups and seemed to connect with one another (Fig. 8). In other instances, obviously epithelial cells lined similar spaces communicating with one another (Fig. 9).

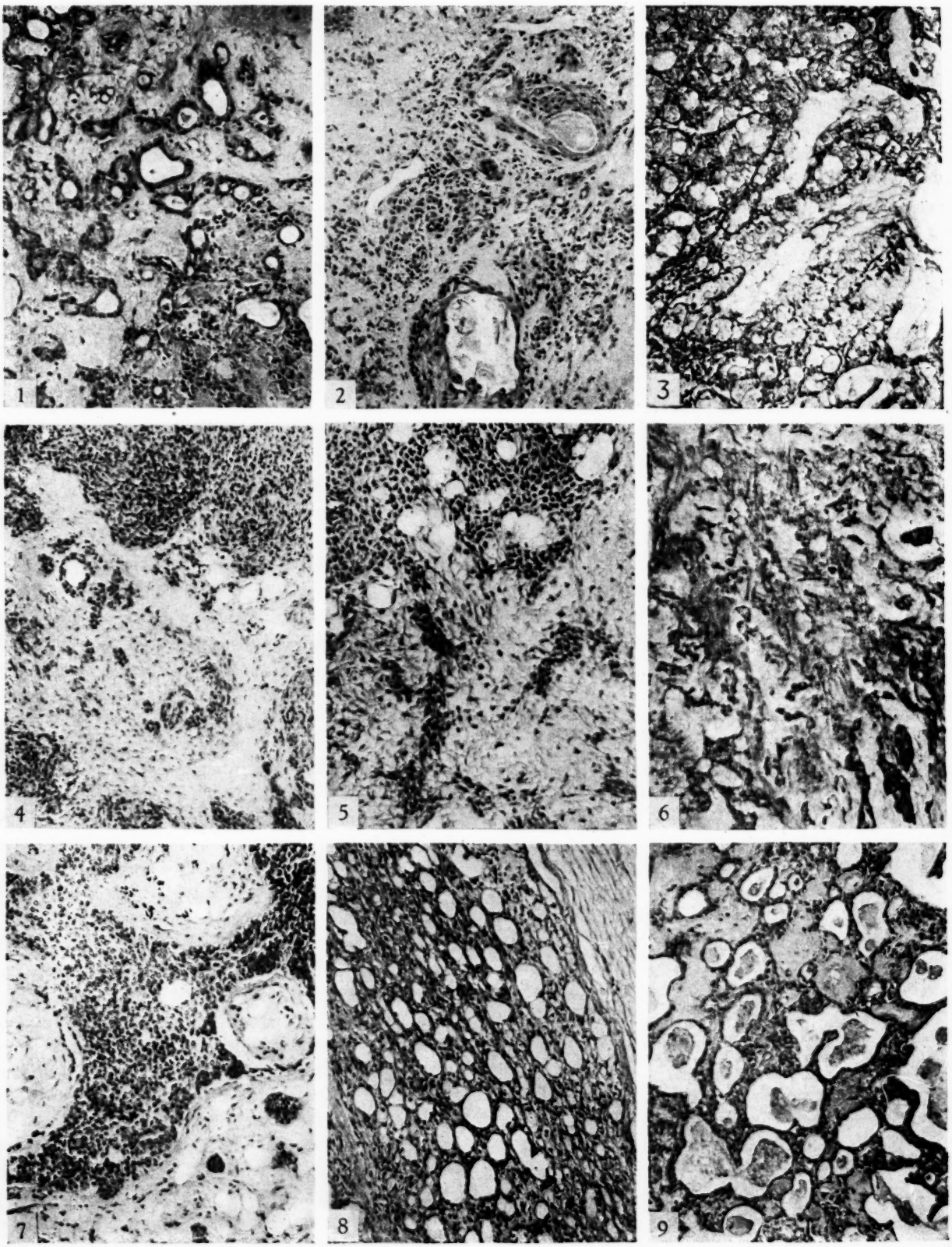
While the growths were usually delimited by a definite capsule, portions occasionally extended into the nearby glandular tissue without disturbing or distorting the pattern of the gland.

COMMENT

Benign growths composed of neoplastic epithelial and neoplastic connective tissue elements usually occur in, near or about the large salivary glands, and are therefore called mixed tumors of the salivary glands. They occur most commonly in or about the parotid glands (1, 6, 12-14, 16, 19), less frequently about the submandibular glands and rarely about the sublingual glands (17). They have also been observed occasionally near the root of the nose, (8), in the nasal septum (7), the palate (15), and pharynx (2), the tongue (5), and the lips (4, 10).

The sites of origin on the face usually correspond to or near lines of fusion of embryonal facial fissures. This circumstance and their structural patterns suggest that, like the adamantinomas and craniopharyngiomas, they are derived from embryonal rests of the ectoderm. The structural differences between the adamantinomas and craniopharyngiomas on the one hand, and the salivary gland tumors on the other, may be attributed to at least two factors. One of these is the influence of the environment on the neoplasms in their respective sites. The other factor is the stage of evolution of the cell rests at the time of separation from the ectodermal anlage.

Adamantinomas and craniopharyngiomas are evidently of more recent genetic origin, hence their simpler range of variation in cellular patterns. The stroma in these growths is a contribution of the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surrounding connective tissue, whereas in tumors of the salivary gland it is a part of the neoplasm (9).

The salivary gland tumors appear to be genetically the oldest of the three. The group of cells from which they are derived must have separated from the ectoderm at the stage when they had as yet not acquired the dominant characteristics as ectodermal or mesodermal cells; hence their capacity to differentiate into both ectodermal and mesodermal tissues. This explains the variations in their individual structural patterns, namely, the presence in varying proportions of undifferentiated and differentiated epithelial elements, of embryonal connective tissue, adipose tissue, endothelial-lined spaces, young and adult connective tissue, hyaline cartilage and osseous tissue. It also explains the apparent transformation of ectodermal elements into mesodermal tissue elements, such as the gradual transition of epithelial cells into cells of hyaline cartilage. It also explains the occasional extension of the growth into the nearby glandular tissue without disturbing or distorting the pattern of the gland.

SUMMARY

The cellular structures of anlage tumors occurring in the salivary glands were studied in 38 patients. In 29, the growth occurred in or about the parotid glands; in 6, about the submandibular glands; and in 3, in the maxilla. There was no fundamental difference in the gross appearance or microscopic structure of the growths arising in the parotid regions and of those situated elsewhere.

The sites of origin and their structural patterns suggest that, like adamantinomas and craniopharyngiomas, they are derived from embryonal rests of the ectoderm. The structural differences may be attributed partly to influences of the environment on the tumors in their respective sites, and in part to the stage of evolution of the cell rests at the time of separation from the ectodermal anlage.

The salivary gland tumors appear to be the oldest genetically. The group of cells from which they originate must have separated from the ectoderm at the stage when they had as yet not acquired their dominant characteristics as ectodermal and mesodermal cells; hence, their capacity to differentiate into ectodermal and mesodermal tissues. The adamantinomas and craniopharyngiomas are evidently of more recent genetic origin; hence, their simpler range of variation in cellular pattern. The stroma in these growths is a contribution of the surrounding connective tissue, whereas in tumors of the salivary glands it is a part of the neoplasm.

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DESCRIPTION OF FIGURES 1 TO 9

Fig. 1.—Sheets of epithelial cells forming no particular pattern are seen in nests and strands in hyaline-like ground substance fading into cartilage. In the larger cell nests, there are various-sized lumina or pseudo-lumina, mostly empty or containing pink amorphous material. (P-25) Mag. × 110.

Fig. 2.—Sheets of epithelial cells form no particular pattern and are in nests, strands, or in stream-like concentric arrangement with clearly discernible intercellular bridges and keratinized round masses forming epithelial pearls. (P-12) Mag. × 110.

Fig. 3—Acinar tubular structures are lined by cuboidal or columnar cells producing a mucinous secretion and are arranged in pattern forming glandular structures. (P-22) Mag. × 110.

Fig. 4.—Fields of embryonal connective tissue composed of cells with stellate processes fade into barely stainable ground substance. (P-29) Mag. × 110.

Fig. 5.—Epithelial cells are scattered in nests or spread apart into single file and are transformed into cells of cartilage. (P-4) Mag. × 110.

Fig. 6.—A mucin-like fibrillar ground substance with scattered groups of epithelial cells fades gradually into young hyaline cartilage. (P-3) Mag. × 110.

Fig. 7.—Scattered groups of adipose tissue cells are seen in the loose connective tissue septa between epithelial cells. (P-2) Mag. × 110.

Fig. 8.—Groups of round or oval empty spaces are lined by flat cells resembling endothelial cells. (P-6) Mag. × 110.

Fig. 9.—Lumina communicating with one another are lined by flat epithelial cells closely resembling endothelial lined spaces. (S-4) Mag. × 110.

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The Changing Cancer Death Rate*

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Epidemiological studies of cancer may be misleading unless individual primary sites are considered separately. Data permitting such analytical investigations were not available in official mortality records prior to 1930, as only 7 classifications of cancer were subdivided by age and sex, except for the years 1914 and 1925. At the present time 30 sites of malignant neoplasms are listed in the vital statistics reports, but even now the cellular variations are not included. Since both the points of tumor origin and the pathologic histology influence the clinical and epidemiological aspects of cancers, consideration of both these factors is essential if one is to avoid the fallacy of mixed classification in the data.

Adjustment of death rates.—Between 1933 and 1944 the crude death rate from cancer for the white population in the Continental United States increased 1.7 per cent annually. The rise was not evenly distributed between the sexes as the rate for females increased 1.2 per cent annually, and that for males, 2.3 per cent. It is impossible to secure a correct picture of changes in mortality that may have occurred in any locality over a period of time unless the effect of a changing age composition in the population is taken into consideration. Therefore, the increased longevity of the population in the United States necessitated an adjustment of the crude death rates in order to compare the rates for a series of years.

When the cancer death rates of the white population for 1933 to 1944 in the Continental United States were adjusted to the age distribution of the total population of the United States, as enumerated in the census of 1940, the annual average increase in the rate for males was 0.76 per cent, while that for females decreased 0.38 per cent annually. These yearly average changes were computed from adjusted rates of all forms of cancer combined, and as the epidemiology of each entity differs the figures fail to portray the true situation. A better knowledge of existing conditions was obtained by studying cancer data by the site of origin. Twenty-two categories were used.

Combined primary sites.—It was impossible to separate deaths from cancer of the stomach from those of cancer of the duodenum for the entire period of observation, but inasmuch as gastric cancer cases represented approximately 98 per cent of the stomach-duodenum group in the years when separation was possible, the inclusion of duodenal cancer throughout this period did not greatly affect the figures and hereafter these cases will be referred to as the "gastric group."

The "buccal cavity group" was comprised of cancers of th lip, tongue, mouth, jaw, and unspecified parts of the area.

In the "other-respiratory group" 80 per cent were cancers of the bronchus; 15 per cent, of the mediastinum and unspecified parts; and the remainder, of the trachea and pleura.

Cancers of the vulva comprised nearly two-thirds of the "vulval-vaginal group."

Of the group consisting of ovaries, fallopian tubes, and parametrium, 99 per cent were ovarian cancers, and hereafter will be called the "ovarian group."

Trends of cancer death rates.—The adjusted rate for each of the 22 sites studied was allocated to one of three classes, depending on whether the adjusted rate showed an upward trend, a downward trend, or no significant trend. The annual percentage of change was obtained for sites with significant trends, using the method described by Gover (4). The class showing downward trends included stomach, buccal cavity, skin, uterus, and liver. In the class exhibiting no significant trend were larynx (female), vulva-vagina, breast, scrotum, bladder, esophagus, kidney (female), and rectum (female). Those in the class showing upward trends were pancreas, testes, prostate, intestines, lung, pharynx, larynx (male), "other respiratory," ovary, rectum (male), kidney (male), and "all others."

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TABLE I: AVERAGE ANNUAL PERCENTAGE CHANGE IN THE ADJUSTED MORTALITY RATES FROM CANCER OF SPECIFIC SITES AMONG WHITE MALES AND FEMALES IN THE CONTINENTAL UNITED STATES, 1933-1944†

SITES	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	Annual Percentage Change
TOTAL CANCER													
Male	106.8	109.8	110.0	112.2	112.6	114.0	115.5	117.4	116.3	115.9	115.7	116.9	+0.76
Female	125.3	127.5	128.1	129.2	126.7	126.4	126.8	125.3	123.3	123.2	123.4	122.7	−0.38
Sites with a downward trend													
Male													
Buccal cavity	6.0	5.9	5.8	5.8	5.5	5.4	4.7	4.6	4.4	4.2	4.3	4.2	−3.8
Skin	4.2	4.0	4.0	3.8	3.7	3.6	3.7	3.7	3.5	3.4	3.1	3.1	−2.4
Stomach	28.3	28.0	27.8	27.4	26.7	26.1	25.1	24.9	23.7	23.6	22.9	22.3	−2.1
Liver	8.3	8.1	7.7	7.6	7.3	7.0	6.8	6.5	6.4	6.4	6.8	6.9	−2.1
Totals	46.8	46.0	45.3	44.6	43.2	42.1	40.3	39.7	38.0	37.6	37.1	36.5	−2.4
Female													
Stomach	19.8	19.4	18.9	18.6	17.6	17.2	16.2	15.2	14.6	14.8	13.8	13.5	−3.8
Buccal cavity	1.3	1.3	1.2	1.2	1.2	1.1	1.0	1.0	0.9	1.0	1.0	0.9	−3.4
Liver	11.3	13.3	10.7	10.3	9.9	9.4	9.0	8.5	8.3	8.5	8.9	8.7	−3.3
Skin	2.2	2.3	2.3	2.3	2.2	2.1	2.1	1.9	1.9	1.8	1.8	1.8	−2.5
Uterus	25.2	25.3	25.3	25.4	25.1	24.1	24.0	23.6	23.0	22.2	22.6	22.3	−1.4
Totals	59.8	61.6	58.4	57.8	56.0	53.9	52.3	50.2	48.7	48.3	48.1	47.2	-2.6
Sites without a significant trend													
Male													
Bladder	5.1	5.2	5.3	5.5	5.2	5.3	5.2	5.3	5.2	5.1	5.0	5.1	*
Breast	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.3	*
Esophagus	3.0	3.1	3.0	3.2	3.4	3.1	3.2	3.3	3.3	3.3	3.2	3.2	*
Scrotum	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	*
Totals	8.5	8.7	8.7	9.0	8.9	8.6	8.6	8.9	8.8	8.7	8.5	8.6	*
Female													
Bladder	2.5	2.4	2.6	2.6	2.6	2.5	2.5	2.6	2.6	2.4	2.4	2.5	*
Breast	22.3	23.1	22.7	23.0	22.8	23.2	23.2	23.6	23.1	23.2	22.9	22.8	*
Esophagus	0.8	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.8	*
Kidney	1.5	1.6	1.6	1.4	1.5	1.5	1.5	1.4	1.5	1.5	1.4	1.5	*
Larynx	0.2	0.2	0.3	0.3	0.3	0.2	0.2	0.3	0.2	0.3	0.2	0.2	*
Rectum	5.2	5.4	5.6	5.6	5.7	5.9	5.9	6.0	5.8	5.6	5.6	5.5	*
Vulva-vagina	1.0	1.0	0.9	1.0	1.0	0.9	0.9	0.9	1.0	0.8	0.9	1.0	*
Totals	33.5	34.6	34.6	34.8	34.8	35.1	35.1	35.7	35.0	34.6	34.2	34.3	*
Sites with an upward trend													
Male													
Other Respiratory	0.6	0.7	0.9	1.3	1.5	1.9	2.6	3.2	3.2	3.6	3.7	3.9	+20.1
Lung	4.0	4.5	5.0	5.2	5.7	5.9	5.8	6.2	6.7	6.9	7.5	8.1	+ 5.8
Pharynx	1.3	1.4	1.3	1.2	1.3	1.3	1.8	1.8	1.9	1.9	2.0	1.9	+ 4.9
Pancreas	3.6	3.8	4.0	4.2	4.3	4.5	4.7	4.7	4.9	4.9	4.8	4.9	+ 2.8
Intestines	10.7	11.5	11.4	11.8	12.2	12.6	13.0	13.0	12.7	13.1	13.6	13.5	+ 2.1
Kidney	1.8	2.0	2.0	2.1	2.1	2.0	2.1	2.3	2.4	2.2	2.2	2.3	+ 1.8
Larynx	1.7	1.7	1.8	1.8	1.8	2.0	1.9	2.0	2.0	2.0	1.9	2.0	+ 1.6
Prostate	11.3	12.1	12.1	12.5	12.7	13.4	13.7	14.0	14.2	13.1	12.9	13.3	+ 1.6
Rectum	6.4	6.6	6.7	6.8	7.4	7.8	7.7	7.8	7.8	7.4	7.5	7.6	+ 1.6
Testes	0.7	0.8	0.7	0.8	0.8	0.8	0.8	0.9	0.9	0.9	0.8	0.8	+ 1.6
All Others	9.4	10.0	10.1	10.1	10.7	11.1	12.5	12.9	12.8	13.6	13.2	13.5	+ 3.6
Totals	51.5	55.1	56.0	58.6	60.5	63.3	66.6	68.8	69.5	69.6	70.1	71.8	+ 3.1
Female													
Other Respiratory	0.2	0.2	0.3	0.3	0.3	0.4	0.7	0.7	0.8	0.8	0.7	0.8	+15.5
Pharynx	0.3	0.4	0.3	0.3	0.3	0.3	0.5	0.5	0.5	0.5	0.5	0.5	+ 5.6
Ovary	4.2	4.8	4.9	5.0	5.1	5.5	5.7	5.9	6.1	6.1	6.0	6.2	+ 3.3
Lung	2.2	2.3	2.5	2.7	2.8	2.7	2.5	2.4	2.6	2.7	3.1	3.0	+ 2.1
Pancreas	3.0	3.0	3.2	3.5	4.4	3.6	3.7	3.6	3.8	3.7	3.5	3.6	+ 1.6
Intestines	13.6	14.2	14.4	14.9	15.0	15.1	15.5	15.0	14.9	14.8	15.7	15.9	+ 1.0
All Others	8.5	6.4	9.5	9.9	9.0	9.8	10.8	11.3	10.9	11.7	11.6	11.2	+ 4.0
Totals	32.0	31.3	35.1	36.6	35.9	37.4	39.4	39.4	39.6	40.3	41.1	41.2	+ 2.5

†Adjusted to age distribution of total U. S. population 1940.
*Not a significant annual change.

The rates for the class showing the upward trend increased annually by 3.1 per cent for males and 2.5 per cent for females; that for the second class showed a downward trend of 2.4 per cent for males and 2.6 per cent for females; whereas there was no significant annual percentage of change in the third class.

Sites with an upward trend in mortality rate.—Among the cancers with an upward trend in rates, those of the lung, "other respiratory," and pharynx showed the greatest increase. Pharyngeal cancer is included with the buccal cavity group in the *Manual of the International List of Causes of Death*, but inasmuch as its trend was at variance with that of the buccal cavity group, it was considered separately Its high rate of increase more closely approaches that of the respiratory group.

The increase in the respiratory group and in that of the pharynx is significant enough to warrant speculation. Part of this apparent increase may be due to more accurate diagnosis. Also, it has been suggested that irritation from respiratory infections and inhaled irritants, including tar dust from the roads, may be of etiological significance (1).

Cancer of the ovary has shown a high rate of increase. A part of this may be the result of improved diagnosis.

Site without significant rate trends.—The adjusted rates for most of the sites in the group without a significant trend were small. The principal exception was the rate for cancer of the breast (female). It is believed that this really belongs with the sites having declining rates, and if there were greater accuracy in the certification of primary breast cancer it would be classified in the downward trend group. A discussion of breast cancer is included in the section immediately following.

Sites with downward trends.—Mortality from cancer of the skin decreased 2.4 per cent annually for each sex, during the period of observation. This is not surprising, since the effects of education and improved methods of treatment would be expected to reduce the deaths from cancer of this accessible site.

Cancer of the buccal cavity showed an even greater reduction than that of the skin, *i. e.*, 3.8 per cent for men and 3.4 for women. Here, too, cancer education and improved therapy are probably major causative factors, but in addition, one must consider the better dental hygiene and nutrition (3) of recent years, the decline in syphilis prior to World War II, and the co-operation of members of the dental profession in urging individuals to seek a medical diagnosis for questionable oral lesions.

Better obsetetrical care, cancer educational activities, and improved cancer therapy are partly responsible for the decrease in the death rate for uterine cancer. A fourth factor may be the declining birth rate, as the relationship between child-bearing and cancer of the uterus is well established. The Cancer Commission of the League of Nations (11) concluded that "it is the fact of a pregnancy and not the number of pregnancies which is the predisposing factor in the production of cancer of the uterus", and since a special report of the Census Bureau on fertility for the years 1910 and 1940 (13) shows an increase in nulliparous women, a decrease in the death rates from cancer of the uterus might be expected.

Primary cancer of the liver is rare in this country. Dr. Shields Warren (14) has stated that "Many cancers metastasize freely to the liver. Among these are cancers of the rectum and the stomach. These two examples, however, are no longer important sources of error as they are practically always recognized in their local manifestations and classified accordingly. However, there are cancers, such as those of the pancreas and breast, which may be overlooked, or treated and forgotten that do contribute to metastasis in the liver." It is probable that the lowered death rate from cancer of the liver is due principally to a more accurate diagnosis of cancers of the pancreas and breast. Of course, cancers from sites other than those mentioned above involve the liver, but there is less probability of their being diagnosed erroneously as cancer of the liver.

If the assumption is made that most of the instances classified as primary cancer of the liver were actually secondary to primary cancer of other organs, it would seem reasonable to apportion and combine them with their sites of origin. It is believed that primary breast cancers comprise more than 50 per cent[1] of those erroneously classified, but this figure has been used in order to be ultraconservative. Fifty per cent of the hepatic cancers were apportioned to the breast and new trends were computed. Cancer of the breast among women then showed a significant downward trend. The downward trend in liver cancer among females probably results from a real improvement in the mortality from breast cancer. If that is so, cancer of this site should be included in the downward trend class,

[1] Among 531 autopsy records of women who had metastases to the liver 36.5 per cent were found to be primary breast cancers. When the sites which might have been erroneously classified on the death records were segregated, cancer of the breast comprised nearly 70 per cent of this group.

rather than in that showing no significant trend. With the increasing number of breast cases treated during the early stages, and with the greater number of 5 year cures, it seems reasonable that an improvement should be reflected in the mortality statistics, and it is believed that this has occurred.

Cancer education and improved medical and surgical service have been considered important factors in the reduction of rates for the sites just discussed. As for cancer of the stomach, however, the situation is different. The decrease that has occurred in the mortality from gastric cancer is far greater than could be expected to result from the general educational programs and from improved medical service. While a small part of this may have been the result of better classification, other factors must have been operating. In looking for an explanation, the findings of Stevenson seem pertinent (10).

In the Registrar-General's Decennial Supplement for 1931, Stevenson analyzed the cancer mortality statistics for England and Wales according to exposed and non-exposed sites; and then these groups were subdivided into 5 classes according to the economic status of the individuals, as determined by occupation. Cancers of the exposed areas showed the highest mortality in the unskilled classes and were classified as Group I. Cancers of the non-exposed sites were classified as Groups II and III, the former consisting of those sites with lowest mortality in the unskilled classes, and the latter, those sites showing no definite relationship between mortality and social class.

Stevenson and his colleagues (9) in reporting on these findings stated: "It thus appears that a large proportion, at least, of cancer mortality is of a highly preventable nature, for we must suppose that if the conditions of life of all sections of society could be assimilated to those of its upper ranks, mortality from cancer of the exposed sites would fall for all classes to the Class I level. Indeed, it is very possible that knowledge of the preventable causes accounting for the difference might provide the means of reducing if not eliminating these forms of cancer for all causes, for these causes might well be found to apply in varying degree to all sections of society."

Stevenson's Group I included cancer of the upper alimentary canal, larynx, skin, scrotum, penis, uterus, and of the vulva-vagina. Most of these sites fall into either the downward trend class of the present study or into that with no trend. It seems improbable that a downward trend in cancer of the stomach could be caused by control measures, although several of Stevenson's Group I sites could be so influenced. It would appear that the stomach might be the site that would best reflect any correlation between lowered incidence and improved standards of living.

The age at which carcinogenic influences have their inception is unknown, but the Kennaways (6) suggest that gastric cancer arising after the second 25 years of life may be predestined to occur by factors to which the body was exposed during the first 25 years. Hueper (5), in discussing occupational cancer, asserts that "The exposure time necessary for eliciting cancerous reactions in exposed workers varies greatly with the type and potency of the carcinogenic agent, with the intensity of exposure and with the susceptibility of the individual. The range is from six months to three or four decades, while the average exposure time is from five to fifteen years."

If the change in the rate for cancer of the stomach began around 1926, as reported by Dublin and Lotka (2), one might expect the changes that caused the decreasing incidence to have occurred about the turn of the century. Lombard and Doering (8), in discussing possible causative factors of gastric cancer, questioned whether irritation could be caused by hasty eating of monotonous, poorly prepared food, and further questioned whether such irritation might be the exciting cause of cancer of the stomach. According to the United States Department of Agriculture (12) ". . . diets have changed in this country in two generations. Analysis shows that the proportion of calories derived from milk, cheese, fruits, and succulent vegetables has actually doubled in 50 years. There has also been a slightly greater emphasis on sugars and fats."

It has been said that the greatest improvement in dietary habits has occurred in the lowest economic group. If the majority of people in the United States are securing a wider variety of properly prepared, nutritious food, one can at least consider the possibility of a real relationship between these factors and a decrease in gastric cancer.

The effect of cancer education.—The decrease in the rates for cancer of the stomach, uterus, skin, buccal cavity and liver was sufficiently great to cause a downward trend in the total female cancer rate. Inasmuch as a part of the improvement has been attributed to education it would be desirable to compare this trend with the one for Massachusetts, where intensive educational work has been of longer duration than for the country as a whole. Unfortunately many variables make such a comparison of doubtful value. A comparison between the trend for Massachusetts and that for the Registration

Area of 1900 would be more satisfactory. Such a study has been made by Lombard (7). "In the early part of the century the adjusted cancer death rates for both sexes were rising about two per cent per year in both the Registration Area of 1900 and Massachusetts. Shortly before this country entered the First World War the increase among females lessened, somewhat more in Massachusetts than in the Registration Area. A change occurred in the male rate around 1926 for both Massachusetts and the Registration Area. From then on the annual percentage increase was only about one-half its previous value. In the middle thirties, a downward trend was noted in Massachusetts among females and a few years later a similar drop occurred in the Registration Area of 1900."

If the assumption is made that the drop in the cancer rate is the result of cancer control activities, the earlier decline in the female rate for Massachusetts seems a logical occurrence, inasmuch as this state was the first to develop an integrated cancer control program. The later drop, which appeared in the trends for the United States, might be expected when one considers the increased cancer control activity that occurred as the result of the inauguration of the cancer program of the United States Public Health Service, that of several states, and the educational activities of the American Cancer Society.

SUMMARY

During the past 12 years the adjusted mortality rates for cancer of the stomach, buccal cavity, skin, uterus and liver have shown downward trends for the white population of the Continental United States. The rates for cancer of other sites, including pancreas, testes, prostate, intestines, lung and "other respiratory" sites, have shown upward trends; whereas for the larynx (female), vulva-vagina, breast, scrotum, bladder, esophagus, kidney (female), and rectum (female) no significant trend was apparent. The sites of origin showing downward rate trends may be divided into 2 categories: those that probbably have been influenced by education and improved therapy, and one site (stomach) in which improvement may be the result of better living conditions.

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Neoplasms in Rats with 2-Acetaminofluorene and Sex Hormones II*

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In a previous communication (4) we reported that administration of estrogen, androgen or gonadotropins intensified and accelerated the development of the cystic and neoplasatic hepatic lesions induced in Sherman rats by 2-acetaminofluorene. The purpose of the present report is to review the incidence and distribution of all neoplastic and other significant lesions in a larger series of animals treated over a longer period.

MATERIAL AND METHODS

Of an original group of 173 rats of the Sherman strain weighing about 75 gm., 80 served as controls and 93 received sex hormones. All received 0.03 per cent 2-acetaminofluorene in the following diet: corn meal, casein, alfalfa, linseed oil, bone ash, and NaCl, with supplements of brewers' yeast and cod liver oil.

Groups 1 and 2 (Controls).—Sixty female and 20 male rats received no additional treatment; 18 females and 7 males died too early to permit their inclusion in the experimental data.

Group 3 (Exogenous estrogen).—Thirty-six female rats received 0.125 mgm. of estradiol dipropionate (in sesame oil) intramuscularly 3 times weekly; 15 survived sufficiently long to permit their inclusion in this report.

Group 4 (Endogenous estrogen).—Twenty female rats received 25 I.U. of pregnant mare serum (PMS) gonadotrophin (in aqueous solution) intramuscularly 3 times weekly; 17 survived sufficiently long to permit their inclusion in this report.

Group 5 (Exogenous androgen).—Twenty male rats received 0.5 mgm. of testosterone propionate (in sesame oil) intramuscularly 3 times weekly; 15 survived sufficiently long to permit their inclusion in this report.

Group 6 (Endogenous androgen).—Seventeen male rats received 25 I.U. of chorionic gonadotrophin (in aqueous solution) intramuscularly 3 times weekly; 9 survived sufficiently long to permit their inclusion in this report.

Treatment was continued in all instances until the time of death or sacrifice.

Tissues were fixed in Bouin's fluid and slides were stained with hematoxylin and eosin. Other slides were stained with Masson's trichrome stain for connective tissue, Marchi's fluid or scarlet red for fat and Best's carmine stain for glycogen (absolute alcohol fixation).

Vaginal smears were made repeatedly during the experimental period and in every instance at the time of sacrifice.

RESULTS

This communication is concerned with the gross and histologic findings in 111 animals examined after 61 to 375 days of treatment. No tumors have been observed in this strain of rats maintained on the basal diet without addition of a carcinogen.

Liver.—The hepatic lesions have been described in detail previously (4). Degenerative and regenerative changes occurred in some degree in all groups, being generally slight in the controls and most pronounced in those receiving testosterone.

Proliferation of young, cellular, connective tissue was observed in all groups; in no instance was it striking, the Masson stain failing to reveal a sharp increase in collagenous fibers.

There were focal abscesses and areas of chronic inflammation in 10 estradiol-treated animals that had pyometra.

Cystic changes were most striking and most constant. The cysts varied widely in size, shape and number, being most conspicuous in animals receiving estradiol. In some instances these were hemorrhagic and occasionally ruptured into the peritoneal cavity. Occasionally, lesions were observed that conformed to that designated as "cyst-adenoma" by Opie (8). This was encountered most frequently in animals receiving chorionic gonadotrophin.

The neoplastic lesions, similar to those induced

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by p-dimethylaminoazobenzene (5, 8, 9), were classified as (a) hepatomas (adenohepatoma and trabecular hepatoma) and (b) cholangiomas. Both occurred in multiple foci throughout the liver. Hepatomas were found in 73 animals, in 9 of which cholangiomas also were present. Their occurrence in the various experimental groups was as follows:

Control females (*Tables I*).—Earliest 265 days; present in 23 of 25 animals examined after 265 to 375 days. Metastasis to lung was noted in 4 cases.

Control males (*Table II*).—Earliest 244 days; present in all of 12 animals examined after 244 to 333 days. Metastasis to lung in 1 and mesenteric lymph nodes in 1 case.

Estradiol (*Table I*).—Earliest 165 days; present in 10 of 14 animals examined after 165 to 263 days.

PMS Gonadotrophin (*Table I*).—Earliest 199 days; present in 9 of 16 animals examined after 199 to 321 days. Metastasis to lung was found in 2 cases.

Testosterone (*Table II*).—Earliest 206 days; present in all of 11 animals examined after 248 to 329 days. Metastasis to lung was found in 2 cases.

Chorionic gonadotrophin (*Table II*).—Earliest 162 days; present in all of 8 animals examined after 162 to 298 days. Metastasis to lung was found in 2 cases and to mesenteric lymph nodes in 1 case.

The liver weights, in grams per 100 gm. of body weight, are indicated in Table III and the pertinent comparative data for different periods of duration of the experiment in Tables I and II.

Lung.—Metastases were found in the lung in 10 (13.7 per cent) of the 73 animals with hepatomas. All occurred late, the earliest (PMS gonadotrophin) at 278 days. The lesions consisted either of minute nodular foci of pale polyhedral cells about or within the lumen of blood vessels, or of grossly palpable nodules replacing the lung tissue, with adjacent areas of hemorrhage.

Metastasis to the lung from adenocarcinoma of the breast occurred in 1 case. Inflammatory lesions were present in the lungs in 3 animals and hemorrhage in 1.

Mammary glands.—Mammary adenocarcinoma (Fig. 1) occurred in 10 (23.8 per cent) of the female controls, none of the estrogen-treated and 3 (17.6 per cent) of the PMS gonadotrophin-treated animals, the earliest after 191 days (control). None was found in male animals.[1] A giant-cell sarcoma (Fig. 6) occurred in the mamma in 1 estrogen-

TABLE I: PRIMARY NEOPLASMS IN FEMALE ANIMALS

	Controls				Estradiol				PMS gonadotrophin			
		Primary neoplasm				Primary neoplasm				Primary neoplasm		
Days of Treatment	No. of rats	Liver	Mammary gland	Bladder	No. of rats	Liver	Mammary gland	Bladder	No. of rats	Liver	Mammary gland	Ear
150-200	10		1		7	5			2	1		
201-250	6		1		5	3	1		3	1		1
251-300	5	4	2		3	2		1	10	5	1	
301-350	10	8	3						2	2	2	
351-400	11	11	3	1								

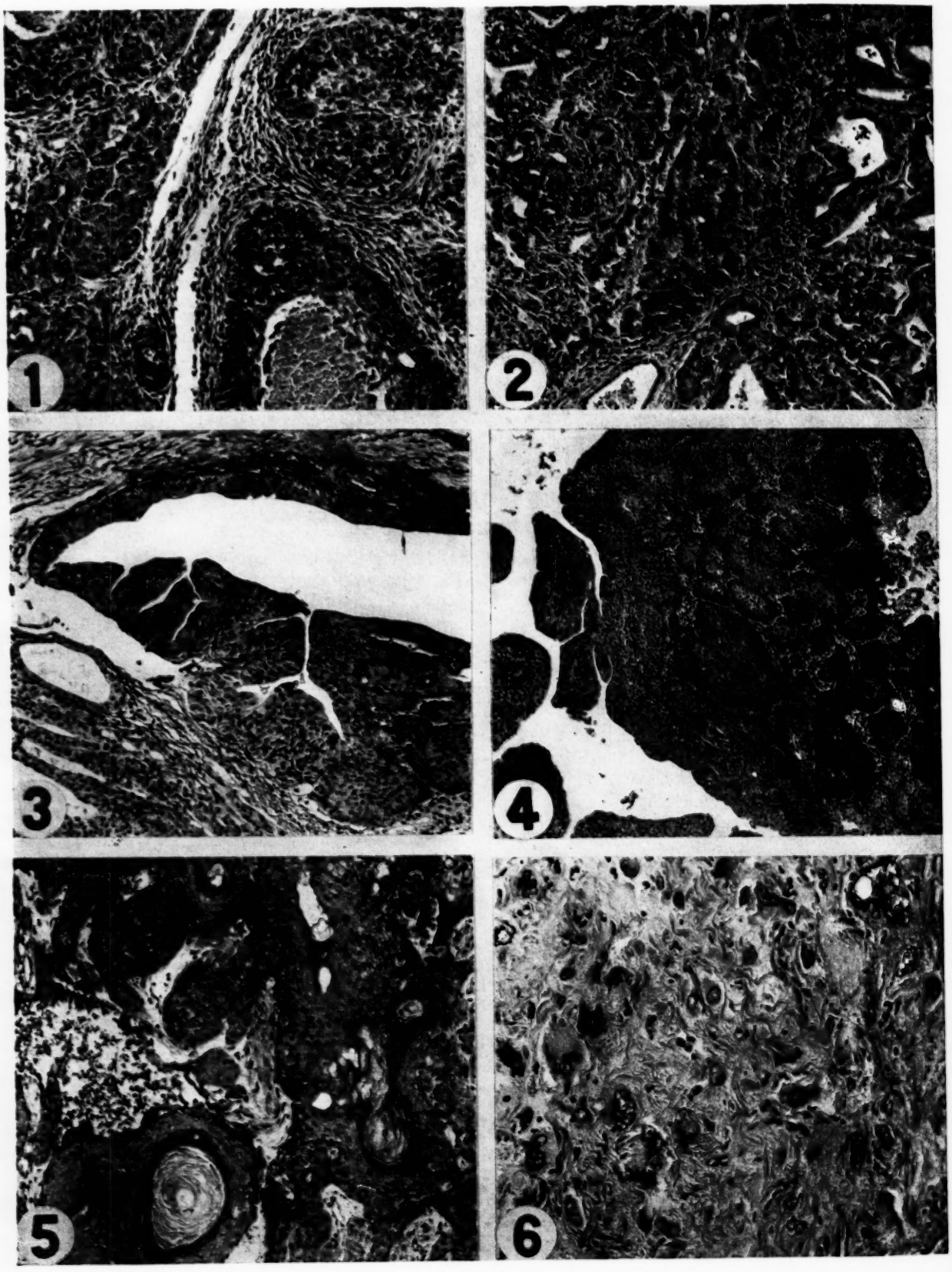
TABLE II: PRIMARY NEOPLASMS IN MALE ANIMALS

	Controls			Testosterone			Chorionic gonadotrophin		
		Primary neoplasm			Primary neoplasm			Primary neoplasm	
Days of treatment	No. of rats	Liver	Bladder	No. of rats	Liver	Bladder	No. of rats	Liver	Bladder
150-200	1						2	1	
201-250	2	2		5	2		3	3	1
251-300	2	2		4	4		4	4	
301-350	8	8	1	6	6	2			

TABLE III: LIVER WEIGHT IN RESPECTIVE GROUPS IN GRAMS PER 100 GRAMS BODY WEIGHT

	Females						Males					
	Control		Estradiol		PMS Gonadotrophin		Control		Testosterone		Chorionic Gonadotrophin	
Days of treatment	Non-tumor, gm. %	Tumor, gm. %	Non-tumor, gm. %	Tumor, gm. %	Non-tumor, gm. %	Tumor, gm. %	Non-tumor, gm. %	Tumor, gm. %	Non-tumor, gm. %	Tumor, gm. %	Non-tumor, gm. %	Tumor, gm. %
150-200	5.6	—	4.3	9.9	7.1	6.3	4.7	—	—	—	11.8	11.1
201-250	6.0	—	5.1	20.0	6.5	8.5	—	8.7	8.9	9.7	—	14.2
251-300	8.5	8.3	18.9	18.3	8.0	15.3	—	8.9	—	24.3	—	22.2
301-350	3.5	17.4	—	—	—	13.5	—	9.8	—	23.1	—	
351-400	—	12.0										

[1] Since tabulation of these data, mammary carcinoma has developed in 1 male rat receiving acetaminofluorene and testosterone for 141 days, and in 2 male rats receiving estradiol dipropionate for 177 and 181 days.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treated animal (6.7 per cent). It should be pointed out that the highest incidence of mammary tumors in the control group occurred after 320 days and that only 2 of the PMS gonadotrophin-treated and none of the estrogen treated animals were maintained for this length of time. Metastasis from these lesions occurred in 2 instances, 1 to the lung, (PMS gonadotrophin) and 1 to the liver (control) (Fig. 2).

The neoplastic lesions in the mamma consisted of single or multiple unilateral or bilateral nodules, varying in shape and size (diameter up to 2.5 cm.). They were irregularly lobulated, grey-brown in color and often cystic, containing hemorrhagic fluid or dark brown, chocolate-like material.

Microscopic examination revealed proliferation of cuboidal or columnar epithelial cells, with basophilic cytoplasm and hyperchromatic nuclei, either forming cystic structures with papillary projections into the lumen, or tending to form acini or tubules. In some areas the stroma consisted of narrow, connective tissue septa, containing numerous fibrocytes, lymphocytes and polymorphonuclear leukocytes. In other areas the connective tissue was more prominent, with variable cellularity, separating groups of epithelial cells.

Female sex organs.—Examinations of the vaginal smears revealed cyclic changes in the control group and estrogenic stimulation in those receiving estradiol. No abnormality was observed in the vagina, uterus, tubes or ovaries in the control group.

Pyometra was present in 10 of the 15 estradiol-treated rats, the uterine horns containing pus and showing evidence of chronic suppurative metritis on microscopic study. The epithelium of the cervix uteri was commonly of the stratified squamous variety, with hyperkeratosis. Chronic salpingitis occurred occasionally and the ovaries were atrophic in 2 instances in this group.

The PMS gonadotrophin group showed no evidence (vaginal smears and uterine sections) of disturbance of the estrus cycle. The tubes were normal. The epithelium of the cervix uteri was frequently stratified squamous, with varying degrees of cornification. The ovaries contained cysts, varying in size and number, lined either by dark-staining cuboidal cells, as seen in maturing follicles or by pale oval or polyhedral cells with foamy cytoplasm.

Male sex organs.—The criteria proposed by Moore (7) were employed in evaluating cytological changes in the testis, epididymis, seminal vesicle and prostate. The presence of heads of spermatocytes in the tubular lumen or among the lining cells was regarded as evidence of mature type of spermatogenesis. The presence of spermatogonia only was considered indicative of an incomplete type of spermatogenesis and the absence of evidence of spermatogenesis was termed atrophy. The epididymis was examined for the presence of sperm and for evidence of hyperplasia. Seminal vesicles and prostate were studied particularly for the height of the epithelial cells, the location of the nuclei and changes in the cytoplasm, differentiating between the anterior (coagulating gland), middle and posterior lobes of the prostate.

The control animals presented no abnormality in the testes, epididymes, seminal vesicles or prostate.

In the testosterone-treated group, the changes in the sex organs were distinctly less pronounced than those usually observed in rats receiving this substance alone for comparable periods of time. Little testosterone effect was apparent in animals examined up to 300 days of treatment; there was comparatively little increase in size or weight of the prostate, seminal vesicles or preputial glands and little or no change in the testes. After longer periods, the testes revealed less active spermatogenesis, and the seminal vesicles and prostate contained numerous dilated acini, lined by flattened, low columnar or cuboidal epithelium, the lumens either being empty or containing pink granular or amorphous material.

The chorionic gonadotrophin group showed no disturbance of spermatogenesis and the Leydig cells were either normal or stimulated. The seminal vesicles and prostate rather consistently contained dilated acini lined by flattened epithelium.

Kidney.—No significant abnormality was observed in glomeruli or renal vessels. The tubular epithelium

DESCRIPTION OF FIGURES 1 TO 6

FIG. 1.—Adenocarcinoma of breast in control rat (carcinogen alone). Mag. × 125.

FIG. 2.—Liver of control rat (carcinogen alone), showing metastasis from mammary carcinoma. Mag. × 125.

FIG. 3.—Squamous-cell carcinoma of bladder in rat treated with chorionic gonadotrophin. Mag. × 60.

FIG. 4.—Papillary squamous-cell carcinoma of bladder in control rat (carcinogen alone). Mag. × 80.

FIG. 5.—Keratinizing squamous-cell carcinoma of external auditory canal in rat treated with PMS gonadotrophin. Mag. × 125.

FIG. 6.—Sarcoma of breast in rat treated with estradiol. Mag. × 160.

showed variable degrees of degenerative change, characterized by swelling and granulation or vacuolization of the cytoplasm, the nuclei being fairly well preserved. The severity of these changes in the control groups was not related to the duration of treatment. Severe tubular degeneration occurred most consistently in the chorionic gonadotrophin group.

Those receiving testosterone and PMS gonadotrophin for prolonged periods showed hyaline and occasional granular casts, in addition to degeneration and dilatation of the tubules.

Urinary bladder.—The transitional epithelium of the bladder was often thickened and stratified, with subjacent infiltration by chronic inflammatory cells. In some cases there were papillary projections into the lumen, lined by transitional or stratified squamous epithelium well-differentiated and with intact basement membrane. Evidence of malignancy was present in 6 instances, the epithelial cells, containing hyperchromatic nuclei, being irregularly arranged and extending beyond the fragmented basement membrane. In 4 of these the cells were squamous in type, with characteristic pearl formations and infiltration into the bladder wall (Figs. 3 and 4).

External ear.—A tumor was present in the external auditory canal in one animal (PMS gonadotrophin). It was a squamous cell carcinoma similar to that described by Bielschowsky (1) as a ductus acusticus tumor (Fig. 5).

DISCUSSION

Our findings differ from those reported by Wilson, DeEds and Cox (10) and by Bielschowsky (1, 3) in the comparatively limited distribution of primary malignant lesions, none occurring in the skin, subcutaneous tissue, muscle, ureter, renal pelvis, intestine, pancreas or lung. We have encountered no tumors of the submaxillary or parathyroid glands (6). Tumors of the thyroid and one of the uterus developed in rats receiving 2-acetaminofluorene and thiouracil; these will be reported elsewhere as will findings referable to the hematopoietic system in the entire series.

In the females treated less than 250 days, mammary carcinoma occurred in 2 of 16 controls (12.2 per cent), in 1 of 12 receiving estradiol (8.3 per cent) and in none of 5 receiving PMS gonadotrophin. It was present in 8 of 26 female controls (30.8 per cent) treated for more than 250 days and in 3 of 12 PMS gonadotrophin-treated animals (25 per cent) during the same period. Although too few animals received estradiol to permit evaluation of its influence upon the incidence of mammary carcinoma after more than 250 days of treatment, the conclusion seems justifiable that exposure to increased amounts of estrogen had no significant effect in accelerating the development of this lesion. No mammary tumor was observed in male rats. Bielschowsky (1) found mammary cancer in 23 of 36 females (63.9 per cent) in 3 of 41 males (7.3 per cent), and in 1 of 11 female castrates (9.1 per cent). This difference is particularly interesting in view of the lack of effect of estradiol noted above.

Bladder tumor occurred in 2 females (2.7 per cent) and 4 males (10.7 per cent), hormone therapy being without apparent influence upon its development. No tumors of the bladder were observed by Bielschowsky (1) whereas it was the most common tumor (25.6 per cent) in the series reported by Wilson, DeEds and Cox (10). It should be noted that the latter encountered it most frequently in animals receiving relatively large doses of carcinogen (0.125 per cent of the diet) and that in our series it occurred after longer periods of treatment than were employed by Bielschowsky.

The comparatively low incidence of liver tumors (28.6 per cent) in control animals in our previous report (4) was due to the large proportion treated less than 250 days. This figure rose to 61.8 per cent (34 of 55 animals) in the present series. Bielschowsky (1) emphasized the higher incidence of hepatic malignancy in male rats receiving 2-acetaminofluorene (82.8 per cent as compared to 30.8 per cent in females). Although this observation is apparently corroborated by our data (54.8 per cent in females, 92.3 per cent in males), the difference may be apparent rather than real. Whereas 16 female controls were examined after less than 250 days of treatment, only 3 males were examined during this period, liver tumors being present in none of the former and in 2 of the latter. This apparent sex difference disappeared after more prolonged treatment, the incidence of this lesion being 88.5 per cent in females and 100 per cent in males treated longer than 250 days. Data must be obtained on a larger number of males treated for 170 to 250 days before a definite conclusion may be reached on this point.

The most striking effect of administration of estradiol and PMS gonadotrophin to females and of testosterone and chorionic gonadotrophin to males was intensification of the cystic and neoplastic hepatic lesions induced by 2-acetaminofluorene. Aggravation of the cystic lesions was most pronounced in the case of animals receiving estradiol, testosterone and chorionic gonadotrophin (4).

Although little can be said regarding the time of first appearance of neoplasms of the liver, their occurrence in less than 250 days in 8 of 12 estra-

diol-treated (66.7 per cent) and 2 of 5 PMS gonadotrophin-treated (40 per cent) females as compared with none of 16 female controls (Table I) suggests a significant influence of these agents in this connection. This is indicated also by a comparison of the liver weights of treated and control females during the first 300 days (Table III), which reflects the difference in extent of hepatic involvement in these groups.

The paucity of data on male rats treated for less than 250 days makes it impossible to evaluate the influence of testosterone or chorionic gonadotrophin in accelerating the development of hepatic neoplasms. However, the striking effect of these agents upon the extent of hepatic involvement is indicated by the enormously greater liver weights after 250 days in the hormone-treated as compared with the control males (Table III).

The hepatic lesions have been described and discussed in detail elsewhere (4). As in the smaller series reported previously the occurrence and extent of the cystic and neoplastic lesions was not paralleled by degenerative, regenerative or fibrotic processes in the liver.

The original purpose of this study was to determine whether hyperplasia of the uterus, ovaries, prostate, seminal vesicles, preputial glands and testes, hormonally induced during a period of exposure to 2-acetaminofluorene, would lead to the development of neoplasms in these organs, as in the hyperplastic thyroid produced by antithyroid drugs (2, 4). No tumors have as yet been found in the target organs of the hormones employed, nor was the development of mammary carcinoma in female rats accelerated by administration of estradiol. However, certain peculiarities have been observed in the action of some of these hormones. Whereas the target organ response to estradiol was comparable to that generally obtained in rats receiving this substance alone, that to testosterone was almost nonexistent in many instances and minimal in the remainder.

The absence of a significant testosterone effect upon the prostate, seminal vesicles, and testes is particularly striking in view of its pronounced effect upon the extent of the neoplastic change in the liver (Table III). The possibility is suggested that, under the existing experimental conditions, the metabolism of testosterone in the liver is so altered as to produce a substance which is carcinogenic or cocarcinogenic but only slightly if at all androgenic. A similar hypothesis may be invoked in explanation of the influence of estradiol upon the development of hepatic malignancy. The maintenance of an estrogenic effect may be attributed to a relatively high estrogenic potency of the hypothetical carcinogenic or cocarcinogenic metabolite or to the escape of a portion of the estradiol into normal metabolic pathways.

SUMMARY

Malignancy of the liver, breast, bladder and external auditory canal occurred in Sherman rats receiving 0.03 per cent 2-acetaminofluorene in the diet for 174 to 375 days.

Mammary carcinoma occurred only in females.[1] Its development was apparently not accelerated by administration of estradiol or PMS gonadotrophin.

Malignant lesions of the liver occurred in 54.8 per cent of females and 92.3 per cent of males receiving the carcinogen alone. Administration of estradiol and PMS gonadotrophin to females and of testosterone and chorionic gonadotrophin to males intensified the cystic and neoplastic hepatic lesions induced by 2-acetaminofluorene. The latent period was considerably shortened in the females and the extent of neoplastic involvement of the liver was increased in both sexes.

No tumors occurred in the target organs of the hormones employed. The target organ response to estradiol was comparable to that generally obtained in rats receiving this substance alone, but that to testosterone was almost nonexistent in many instances and minimal in others. The possible significance of these findings is discussed.

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Heavy water and deuterium gas are now being made available by the United States Atomic Energy Commision for research purposes within the United States. The abundance of the heavy hydrogen isotope in the material is approximately 99.8 per cent. Quantities will be limited to normal research requirements.

The Stuart Oxygen Company of San Francisco, California will act as contracting agent for distribution. This company began small-scale production and distribution soon after the discovery of deuterium. In the post war period, because of the increased need for deuterium in research, the Stuart Company's production has been far below the demand. The quantities being made available are from stock produced by other operators during the war for the Manhattan District.

The material will be distributed with charges based on the cost of handling and distribution. The cost of production of the material itself will not be included. Distribution in this manner is being effected under authority of the Atomic Energy Act of 1946 which provides for the fostering and assistance of research by the Commission.

Allocation will be handled in a manner similar to that for radioisotopes. For complete information write to U. S. Atomic Energy Commission, Oak Ridge, Tennessee, Attention: Isotopes Branch.

Germinoma of the Pineal

Its Identity with Germinoma ("Seminoma") of the Testis

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In 1944 Dorothy Russell (15) published the results of a study of pineal tumors. She presented a rational explanation for the well known similarity in histologic appearance of "pinealomas" and "seminomas." She suggested that many "pinealomas" were in truth teratoid tumors. The present report proposes to confirm her observations and to extend her interpretations in accord with the teratologic concepts gained through study of nearly 1,000 tumors of the testis at the Army Institute of Pathology (6).

The files of the Institute contain pathologic material from 23 patients with tumors of the pineal or ectopic "pinealomas." Fifteen tumors were submitted by military installations[1] (Group 1), and 8 were obtained from civilian sources[2] (Group 2). The essential data in all 23 cases are listed in Table I.

Seven of the 15 tumors in group 1 were identical with the testicular tumor termed "spheroidal cell carcinoma," or "atypical teratoma," by Russell (15) and called "seminoma" by many pathologists; this neoplasm is identical with the disgerminoma of the ovary. Because this type of new growth is probably made up of germ cells, possibly even of primordial germ cells, the designation "germinoma," which has been suggested (6) for the tumor which occurs in the gonads, is equally appropriate for that which appears in the pineal. Two neoplasms in group 1 were teratomatous tumors; 1 was an adult teratoma, and the other was a teratocarcinoma which also had germinomatous areas. Although there were 6 neural tumors in the group, only 2 presented the biphasic cellular mosaic of the classic pinealoma.

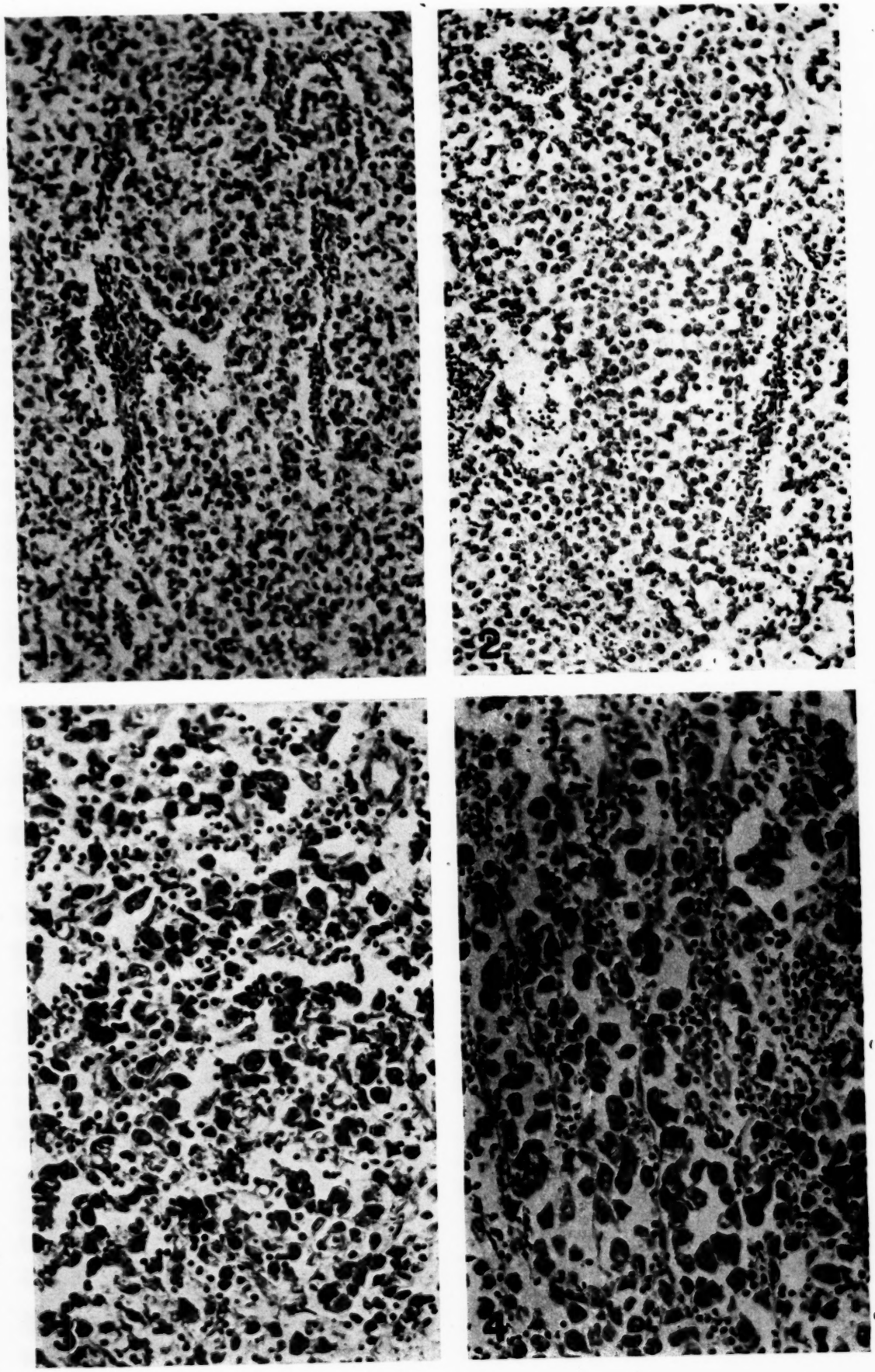
Of the 8 neoplasmas in group 2, 3 were germinomas and 3 were teratocarcinomas. One of the latter also contained both chorioepitheliomatous and germinomatous elements. Only 2 tumors in this group of 8 appeared to be of neural origin; one, which had the pattern of a classic pinealoma, was

TABLE I: DATA IN TWENTY-THREE CASES OF PINEAL NEOPLASM

Case No.	Sex	Age, years	Type of neoplasm	Special features
			GROUP 1	
1	M	29	Neural	
2	M	22	Germinoma	Extrapineal. Pituitary involved. Diabetes insipidus. Hypogonadism.
3	M	17	Neural	
4	M	18	Germinoma	Pituitary involved. Diabetes insipidus.
5	M	21	"	Pulmonary metastases. Radiosensitivity.
6	M	23	"	Pituitary involved. Diabetes insipidus.
7	M	18	"	Pituitary involved.
8	M	28	"	" "
9	M	24	Neural	
10	M	30	"	
11	M	26	Germinoma	
12	M	28	Neural	
13	M	—	"	
14	M	34	Teratocarcinoma	Pulmonary metastases.
15	M	26	Teratoma	
			GROUP 2	
16	F	22	Germinoma	Extrapineal. Pituitary involved. Diabetes insipidus. Polyglandular insufficiency.
17	M	18	"	Spinal metastasis.
18	M	10	Teratocarcinoma	Pubertas praecox
19	M	13	Germinoma	
20	—	—	Teratocarcinoma	
21	—	—	"	
22	F	7	Neural	
23	M	10	"	Pubertas praecox

[1] Cases 5 and 14 are being reported in detail by Tompkins, Campbell and Haymaker.

[2] Material supplied from the laboratories of Dr. Shields Warren, Dr. Lawrence W. Smith, Dr. Norman Elton, Dr. Percival Bailey, Dr. Joseph H. Globus, and Dr. Abner Wolf.



diagnosed by Bailey (3), at the seminar of the American Society of Clinical Pathologists in 1943, as a pinealoblastoma. The tumor presented at that same meeting as a pinealoma falls into the germinoma category.

Of the entire group of 23 tumors, 10 were germinomas and 5 were teratomas or teratocarcinomas. If this group can be considered representative, approximately 2 of every 3 neoplasms of the pineal are not neural tumors.

In Figs. 1, 2, 3 and 4 the histologic appearance of representative "pinealomas" (pineal germinomas) is compared with that of testicular neoplasms of the germinoma type. It is remarkable that all the patterns encountered in germinomas of the testis can be matched in the pineal tumors of this type. For example, the pineal germinoma in case 17 showed a granulomatous reaction (Fig. 5) such as is commonly seen in both testicular and ovarian germinomas; similar granulomatous stroma and giant cells are evident in the "pinealomas" in Figs. 21, 27 and 29 of the report by Globus and Silbert (8). Some germinomas of the gonads and the pineal lack "lymphoid stroma" altogether; others form pseudotubular structures. Finally, the cytoplasm of the principal cells may be either clear or darkly stained.

On the basis of the published illustrations, one may conclude that the tumors in cases 1, 2, 3 and 4 of Globus' 1941 report (7), those in cases 4 and 7 of the 1925 study by Horrax and Bailey (11) and the neoplasm in case 2 of Baggenstoss and Love (2) are germinomas.

In view of the morphologic identity of the two types of neoplasms, it is interesting that they sometimes exhibit a similar sensitivity to irradiation. The testicular germinoma ("seminoma") is a notoriously radiosensitive neoplasm; in one case in group 1 striking regression of a pineal germinoma took place after irradiation, a course of events which has been noted in other instances of "pinealoma" (1, 10).

The fact that two different biphasic tumors, the pinealoma and the germinoma, occur in the same tiny organ has led to much confusion. However, Figs. 6, 7 and 8 show the striking differences in cellular size, structure and arrangement of the true pinealoma and the pineal germinoma which Russell (15) emphasized.

The structural patterns observed in the teratomatous tumors of the pineal are comparable with those seen in testicular tumors. For example, chorionepithelioma and combinations of teratoma and germinoma (Fig. 9) or embryonal carcinoma and germinoma as well as typical teratocarcinoma were encountered. The germinomatous tissue observed in pineal teratomas in the past has usually been incorrectly considered a focus of "pinealoma." Even some new growths of the pineal which appear frankly teratomatous in photomicrographs (Horrax and Bailey [12], case 1, Figs. 3 and 5; Baggenstoss and Love [2], case 4), have been classified as neurogenic tumors.

There is no universally acceptable explanation for the development of both the gonadal and the extragenital teratomatous tumors. One may postulate that neoplastic development of the germ cells normally present in the testis and ovary gives rise to the teratomas of those organs. It is more difficult to explain the origin of the extragenital growths. Such neoplasms may develop from displaced germ cells or arise from somatic cells which have retained some embryonic potencies, a hypothesis which Schlumberger (16) recently employed in attributing mediastinal teratomas to dislocations of tissue during embryogenesis.

The theory that the primordial germ cells are segregated from the somatic cells early in embryonic life and migrate through certain tissues before arriving in the gonadal region affords a logical explanation for the origin of the extragenital teratomas and germinomas. The possible importance of such wandering germ cells is emphasized by the fact that ectopic "pinealomas," usually of the germinoma type, have been found in the third ventricle and other structures near the pineal (case 2, group 1; case 16, group 2; Russell [15]; Ford and Muncie [5]). However, no explanation for the localization of germ cells in the pineal region has been advanced. Swift (17) described primordial germ cells in the

DESCRIPTION OF FIGURES 1 TO 4

FIG. 1.—Germinoma of pineal; compare with germinoma of testicle in Fig. 2. This pattern, in which sheets of uniform neoplastic elements are traversed by trabeculae of connective tissue containing lymphoid cells, is common (case 19). Mag. × 160.

FIG. 2.—Germinoma of testicle; compare with tumor of pineal, shown at same magnification, in Fig. 1. Mag. × 160.

FIG. 3.—Germinoma of testicle; compare with neoplasm of pineal in Fig. 4. Intimately intermingled lymphoid elements and neoplastic cells make up one pattern encountered in both pineal and testicular germinomas. Mag. × 250.

FIG. 4.—Germinoma of pineal; compare with photomicrograph, taken at same magnification, of testicular tumor in Fig. 3 (case 7). Max. × 250.

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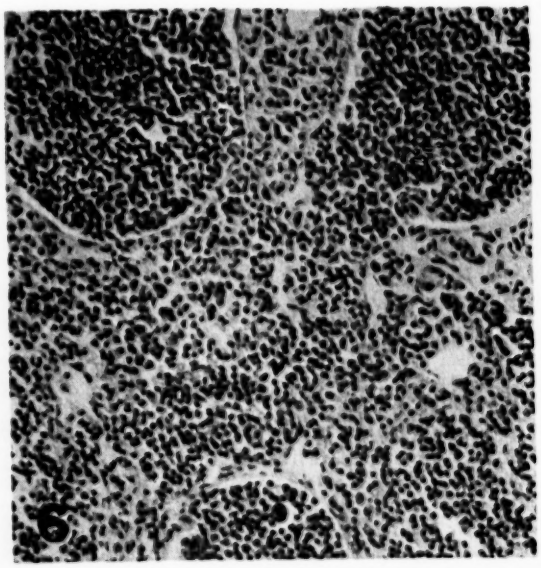
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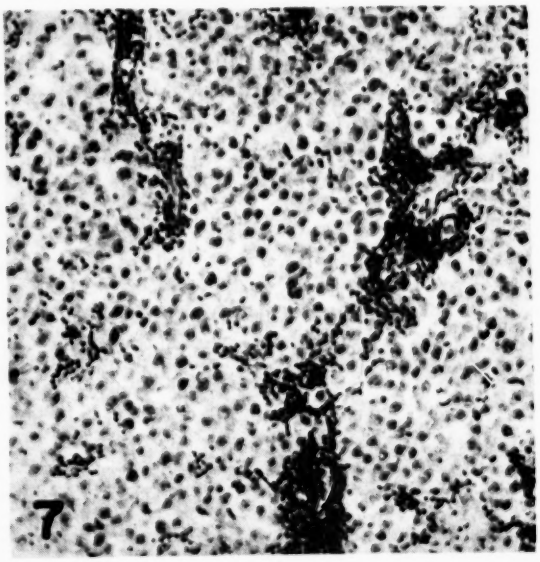
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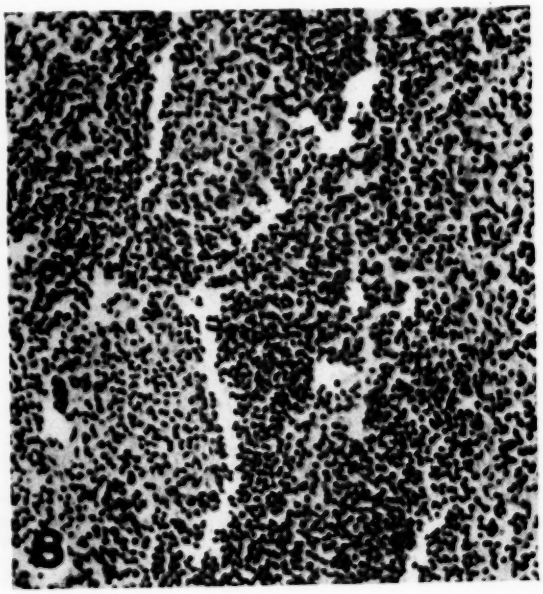
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head region of a chick embryo of 25 somites, and his paper includes a picture showing a group of such cells in a vessel immediately adjacent to the developing forebrain. However, such primordial germ cells move caudad and reach the genital ridge via the mesentery, and it appears reasonable to assume that tumors originating from such elements would develop in the caudal periaortic mesenchyme (9, 13) rather than in the pineal. Because of the route taken by the primordial germ cells their inclusion in Rathke's pouch, which might afford a means of entrance to the central nervous system, also appears unlikely.

The alternative possibility, that somatic cells with undeveloped potencies are included in the developing pineal gland and later give rise to teratomatous growths, has already been advanced. Krabbe (14) stated that the fact that the "germ of the gland" came near the surface of the developing embryo facilitated the penetration of elements of foreign tissue. Charlton (4), in discussing a teratoid tumor of the pineal in a mackerel, expressed the opinion that the neoplasm arose from "mesectoderm" and pointed out that the pineal develops near the anterior neuropore, where ectoderm and neurectoderm meet. Although the pineal actually develops farther caudad than Charlton implied, it may be significant that the sacrococcygeal teratomas occur in the region of the posterior neuropore and neurenteric canal.

Until embryologic investigation supplies a satisfactory explanation of the genesis of pineal germinomas and teratomas, the point of view of the pathologist must remain that stated in 1932 by Harris and Cairns (10): ". . . we are not able to exclude the possibility that this pineal tumour is a tumour of toti-potent cells. It may be more than coincidence that both the pineal gland and the testis . . . which are known to give rise to teratomas, also give rise to spheroidal celled tumours that bear a strong resemblance to one another."

SUMMARY

Study of 23 neoplasms of the pineal collected at the Army Institute of Pathology confirms the view, expressed by other workers, that many so-called pinealomas are identical with certain tumors of the testicle. It is suggested that such new growths may arise from primordial germ cells and should be called germinomas. This hypothesis would account for the relative frequency of teratomas in the pineal. Only 1/of 3 tumors of the pineal originates from neural elements.

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DESCRIPTION OF FIGURES 5 TO 9

Fig. 5.—Germinoma of pineal. Epithelioid cells in lower portion of photomicrograph form a granuloma similar to those encountered in germinomas of testis and ovary. (case 17). Mag. × 250.

Fig. 6.—Pinealoma with classic biphasic cellular mosaic comparable to pattern seen during normal morphogenesis of pineal. Compare size and structure of cells with those of pineal germinoma, shown at same magnification in Fig. 7 (case 1). Mag. × 160.

Fig. 7.—Germinoma of pineal; compare size of cells with those of true pinealoma, shown at same magnification, in Fig. 6. Small lymphoid cells in germinomas are separate, distinct components and show no transitions to large neoplastic elements (case 17). Mag. × 160.

Fig. 8.—Pinealoma showing transitions between 2 cellular components of mosaic; the 2 cell types do not merge in germinoma. Photomicrograph was taken at same magnification as Figs. 6 and 7 (case 22) Mag. × 160.

Fig. 9.—Teratocarcinoma of pineal. In upper right portion of photomicrograph is germinomatous area such as is occasionally encountered in teratocarcinoma of testicle (case 21). Mag. × 60.

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The desperate and continued need for American publications to serve as tools of physical and intellectual reconstruction abroad has been made vividly apparent by appeals from scholars in many lands. The American Book Center for War Devastated Libraries has been urged to continue meeting this need at least through 1947. The Book Center is therefore making a renewed appeal for American books and periodicals—for *technical and scholarly books and periodicals in all fields* and particularly for *publications of the past ten years*. We shall especially welcome complete or incomplete files of *Cancer Research* and the *American Journal of Cancer*.

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Therapeutic Use of Anti-Reticular Cytotoxic Serum (A.C.S.) in Hodgkin's Disease

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In 1920 Metchnikoff (7) expressed the idea that a tissue could be stimulated by injection of small doses of a serum specific for the tissue. Although Metchnikoff's attempt to stimulate erythropoiesis and leukocytosis in animals with small doses of a homologous serum failed, Bogomolets (3), in 1934, apparently solved the difficulty by using the complement fixation reaction to control the titer and dosage of the cytotoxin. Following this discovery several investigators during the past decade have tried to stimulate the reticuloendothelial system in human beings and animals by injecting small doses of a homologous anti-reticular cytotoxic serum (4-11).

Whether the reticuloendothelial system of a human being or animal plays an important role in the defense mechanism against malignant diseases is still far from solved. Recently a group of Soviet scientists reported on the beneficial effect of the anti-reticular cytotoxic serum (A.C.S.) in cancer patients (4), especially when it was used as a prophylactic agent against recurrences of a malignant neoplasm after removal of the primary tumor. In this country a group of investigators (1, 2, 8-10) reported that homologous A.C.S. is capable of inhibiting the growth of tumor cells *in vitro*. Straus (12-14) studied the effect of A. C. S. on the healing of experimentally produced fractures in rabbits. He reported that stimulation of the healing of experimentally produced fractures in rabbits was induced with small "stimulating" doses of A.C.S. and depression of healing was produced with large "depressing" doses. These results correspond to the claims of the Soviet investigators. Bogomolets and Marchuk (4,-6) have reported favorable effects of homologous A.C.S. on the genesis, survival and metastases of induced carcinoma in animals. Shimkin (11) has reviewed these reports and summarized the results obtained with A.C.S.

The study which we undertook was to determine the therapeutic value, if any, of anti-reticular cytotoxic serum in a disease specifically involving the reticuloendothelial system, such as Hodgkin's disease. Hodgkin's lymphogranuloma is regarded as a malignant type of reticuloendotheliosis of unknown etiology.

MATERIALS AND METHODS

The antigen used in the preparation of the anti-reticular cytotoxic serum in rabbits was made from spleen and bone marrow from human cadavers not more than 12 hours post mortem. No material was used from cancerous or infectious cases. The only material suitable for this purpose is from bodies of normal persons killed in accidents or dead from heart failure.

Antigen for the preparation of A.C.S. was prepared with a clean but not aseptic technic. The antigen was freshly prepared for each injection.

Repeated injections of progressively increasing doses of the antigen were made into rabbits according to the technic described by Marchuk (6). The peak of antibody formation was not reached until the fourth to seventh day after the last injections. It is very important that the antigen be fresh.

Twenty-two patients with Hodgkin's disease proved by histologic examination of tissue obtained at biopsy were chosen for study; 13 were females, 9 were males. The age of each individual and the duration of symptoms before treatment with A. C. S. are indicated in Table I. Of the 22 patients selected for study, 2 had received no previous therapy and 20 had previous x-ray therapy. In 5 patients x-ray therapy was used concomittantly with A.C.S., and in 17 cases A.C.S. alone without any other type of treatment was used.

A careful evaluation of the clinical status and hematologic equilibrium was made prior to the beginning and at the end of the therapeutic period of each treatment. A treatment consisted of 3 injections at intervals of 72 hours. For use 0.4 cc. of undiluted serum is diluted with 4 cc. of normal saline and injected subcutaneously as follows:

First injection, first day, 0.5 cc.
Second injection, fourth day, 1 cc.
Third injection, seventh day, 1.5 cc.

TABLE I: LIST OF CASES AND SUMMARY OF TREATMENTS

Case	Age	Sex	Length of illness prior to A.C.S. treatment, years	How many series of injections were given*	Previous x-ray therapy	X-ray therapy combined with A.C.S.
1	27	F	5	6	Yes	No
2	28	F	2	6	"	Yes
3	20	F	1	5	"	"
4	50	M	15	4	"	No
5	30	F	2	3	"	"
6	70	F	2	3	"	Yes
7	30	F	2	6	No	No
8	70	F	2	4	Yes	"
9	35	F	5	5	"	"
10	50	M	1	4	"	"
11	17	M	1	3	"	Yes
12	32	F	2	2	"	No
13	28	F	1	1	"	"
14	28	M	3	8	"	"
15	35	M	3	4	"	"
16	24	F	3	3	"	"
17	28	M	2	4	"	"
18	28	F	8	2	"	Yes
19	24	M	2	4	No	No
20	20	M	10	1	Yes	"
21	60	M	2	1	"	"
22	15	F	2	6	"	"
Average value	34	13F 9M	3½ yrs.	4 treatments	20 yes 2 no	5 yes 17 no

*A series (treatment) means 3 injections 3 days apart. Usual interval between series is 6 weeks.

RESULTS AND DISCUSSION

Ten of the 22 patients who were treated with A.C.S. showed an improvement in their sedimentation rate. This was characterized by a progressive decrease and final return to physiologic limits. These 10 patients were treated by A.C.S. alone.

Increase in the hemoglobin level was observed in 11 cases. In a majority of cases a rise in the number of white blood cells, including lymphocytes and monocytes, was recorded. The 5 cases treated simultaneously by A.C.S. and irradiation did not show the improvement in the hemoglobin level and the mono-lymphocytosis that was observed in the 11 patients treated by A.C.S. alone. Nevertheless the leukopenia or lymphopenia so frequently observed during x-ray therapy was avoided by the treatment with A.C.S. during irradiation. No changes were recorded in the circulating platelets. (Table II).

TABLE II: COMPARISON OF HEMATOLOGIC VALUES OBTAINED AT BEGINNING AND AT END OF A.C.S. THERAPY IN 22 PATIENTS WITH HODGKIN'S DISEASE

	Decrease (cases)	Increase (cases)	Unchanged (cases)
Sedimentation rate	10	0	12
Hemoglobin	0	11	11
White blood cells	0	13	9
Lymphocytes	0	13	9
Monocytes	0	12	10
Platelets	0	0	22

In 12 patients gain of body weight was recorded. (Table III). The average gain was about 6 pounds within 3 months after the beginning of the treatment. In a majority of these cases gain of body weight was accompanied with changes in the hematologic values as recorded above.

TABLE III: COMPARISON OF BODY WEIGHT, RADIOLOGICAL FINDINGS AND LYMPHADENOPATHY AT BEGINNING AND AT END OF A.C.S. THERAPY

	Increase (cases)	Decrease (cases)	Unchanged (cases)
Body weight	12	0	10
X-Ray findings	0	1	21
Lymphadenopathy	0	0	22

One case only showed on x-ray examination a decrease in the retroperitoneal tumor. No other roentgenographic changes in the involved organs were recorded during or after A.C.S. therapy in the other patients (Table II).

Temporary shrinkage of the superficial lymphadenopathy occurring in a few patients cannot be correlated with the A.C.S. therapy and is probably a function of the rate of activity of the disease at that time. Five of the 22 patients who received radiation during or after cessation of A. C. S. therapy responded favorably to the former.

SUMMARY AND CONCLUSIONS

Anti-reticular cytotoxic serum used in the treatment of 22 selected patients with Hodgkin's disease under the dosage and conditions described did not prove to be a curative agent in this disease.

Improvement in the general condition in a majority of the cases treated, such as gain in body weight, favorable changes in the sedimentation rate and blood morphology, were noted.

A.C.S. may be considered a valuable adjunct to other accepted methods of therapy in the treatment of Hodgkin's disease.

ACKNOWLEDGEMENT

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The Effect of Ultraviolet Irradiation on the Carcinogenic Potency of Certain Hydrocarbons*

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As the results of published investigations on concurrent application of carcinogenic agents and irradiation with ultraviolet, visible and infrared light (2, 6-8, 10-19) differ considerably, we have found it of interest to investigate the activity of different carcinogenic hydrocarbons after ultraviolet irradiation.

Cook and Martin (5), Dufraisse and Gerard (9) and Velluz (20) have shown that ultraviolet irradiation of carcinogenic hydrocarbons causes the formation of oxides that have the character of peroxides. Methylcholanthrene and 9, 10-dimethyl-1,2-benzanthracene are more readily photo-oxidized than 1, 2, 5, 6-dibenzanthracene and 3,4-benzyprene.

Cook and Martin (5) have shown that the photo-oxide of 9,10-dimethyl-1,2-benzanthracene is non-carcinogenic in mice. Boyland and Boyland (4) found that the alkali-soluble product of 1,2,5,6-dibenzanthracene after ultraviolet irradiation was non-carcinogenic in mice whereas Allsopp (1) found that the alkali-soluble product of 3,4-benzpyrene after ultraviolet irradiation was carcinogenic in mice.

MATERIALS AND METHODS

In our investigation the hydrocarbons were dissolved in benzene and placed for 72 hours in open Petri dishes at a distance of 50 cm. from an air-cooled 350 watt mercury quartz lamp. After evaporation of the solvent the irradiation products were dissolved in ground nut oil for subcutaneous injection (dose 0.5 mgm. in 0.2 cc. of oil) and in benzene (0.5 per cent) for painting. Mice of the Street strain were used in all our experiments.

The irradiation products thus obtained are, from a chemical viewpoint, by no means pure compounds, but it can be supposed that these "crude mixtures" are in fact products of the same process (photo-oxidation?), as occurs on the skin of mice after concurrent application of ultraviolet light and carcinogenic hydrocarbons. Figs. 1 to 4 give the absorption curves of the compounds used.

1,2,5,6-Dibenzanthracene.—The absorption spectrum of this comparatively weak carcinogenic hydrocarbon is, as shown in Fig. 1, completely altered by irradiation since the irradiation products have lost their characteristic absorption band. Nonetheless, its carcinogenic power is reduced to only a quarter or even half that of the original hydrocarbon.

In the control group of animals painted with nonirradiated hydrocarbon, the first tumor appeared after 9 months. In the 14 mice surviving the 9 months, 7 instances of papillomas or carcinomas were found; whereas in the 20 mice painted with the irradiated hydrocarbon, only 5 tumors appeared, and strangely enough 2 of these were subcutaneous sarcomas at the site of application. One case of spontaneous mammary cancer appeared in both the experimental and control groups, the latter case being complicated by leukemia. In the last group there appeared also one case of gastric ulcer with initial cancer development.

Of the 26 control mice subcutaneously injected with 1 mgm. of hydrocarbon, 17 developed sarcomas (approximately 65 per cent); 1 of these was complicated by leukemia. Besides these, 1 case of mammary cancer occurred. Four cases of sarcoma (approximately 15 per cent) appeared in the group of 27 animals injected with the irradiation product. In this same group there arose 2 cases of mammary cancer (1 complicated by pulmonary adenoma), and 1 isolated case of pulmonary adenoma.

Boyland found that the alkali-soluble irradiation product of 1,2,5,6-dibenzanthracene was noncarcinogenic. It is not stated, however, how many mice are included, nor is the dose of the photo-oxide given. In our investigations the carcinogenic potency of

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the crude mixture of irradiation products showed the reduction to be only 25 to 50 per cent. This may be explained by assuming that the mixture contains unaltered hydrocarbon.

3,4-Benzyprene.—From Fig. 2 it is seen that 3,4-benzpyrene loses its characteristic absorption band after irradiation, although the absorption intensity is not reduced to anything like the same degree as that of the irradiation product of 1,2,5,6-dibenzanthracene.

Among 21 animals painted with nonirradiated 3,4-benzpyrene, 20 tumors (9 papillomas and 11 carcinomas) were found. One of the carcinomas was complicated by leukemia. Of 22 animals painted with irradiated benzpyrene, however, only 11 developed tumors (3 papillomas and 8 carcinomas). The carcinogenic potency is thus reduced by nearly 50 per cent.

In 20 animals treated by subcutaneous injection of nonirradiated hydrocarbon, 7 cases of sarcoma were seen as compared with only 2 cases of sarcoma from 24 mice treated with irradiated benzpyrene.

One case of leukemia and 1 of pulmonary adenoma appeared in the control group; and among the animals treated with irradiated benzpyrene, 1 case of pulmonary cancer was observed.

Thus the difference in the effect is very pronounced. However, the irradiation product of this hydrocarbon, too, possesses no appreciable carcinogenic potency.

The reduction in the carcinogenic potency of 3,4-benzpyrene after irradiation is similar to that of 1,2,5,6-dibenzanthracene; namely, to about 50 per cent by painting and to 25 per cent by subcutaneous injection.

9,10-Dimethyl-1,2-benzanthracene.—By irradiation, the characteristic absorption band disappears and the absorption curve is depressed considerably (Fig. 3). From other investigations it is known that in mice of the Street strain 9,10-dimethyl-1,2-benzanthracene causes cancer in nearly 100 per cent of the animals. In the groups painted with the nonirradiated hydrocarbon, however, only a few tumors occurred because the very toxic effect of the vigorous treatment, the violent epilation, and emaciation caused the death of all animals within the first 3 to 4 months. Of 23 animals only 8 cases of papilloma and 2 of carcinoma were seen. In addition, we found 3 instances of leukemia and 1 of carcinoma in the neck, probably originating from the salivary gland.

Rather strange results were observed by painting with the irradiated hydrocarbon. After 4 to 5 months, 8 cases of small, rather firm, papilloma-like nodules at the site of application appeared in a group of 22 mice. The nodules grew to about 2 to 3 mm. and remained unaltered for some time, as long as 8 months. Three of the nodules regressed spontaneously after 1½, 3, and 7 months; 1 remained unaltered until the animal died at 11 months and 1 became ulcerated after 6 months without showing any sign of malignancy. In 3 other animals malignant tumors developed (1 carcinoma in a 12 month old mouse and 2 sarcomas). On gross examination the carcinoma could not be distinguished from the papillomas (Fig. 5), but microscopical examination revealed a small, well defined, squamous cell carcinoma (Fig. 6). The 2 sarcomas developed at the site of application beneath the papillomas when the animals were 11 and 12 months old. Furthermore, there were 4 cases of gastric ulcer, 2 of which were complicated by carcinoma.

Painting with irradiated 9,10-dimethyl-1,2-benzanthracene has thus a quite different effect from that of nonirradiated hydrocarbon, partly because the irradiation product is not toxic to the same extent as the nonirradiated hydrocarbon that killed all the animals within 5 months, and partly because the carcinogenic potency has been altered considerably. The change, however, seems to be of a somewhat different nature from that of the other hydrocarbons mentioned above. In the latter, (dibenzanthracene and benzpyrene) irradiation effected a certain reduction of the carcinogenic power, but the tumors that appeared were of the same type as those produced by application of the original hydrocarbon. The effect upon the skin of the irradiated 9,10-dimethyl-1,2-benzanthracene is a development of minimal papillomas, which, in spite of continued painting, regressed in most cases or remained unchanged for months. Only 1 minimal, well defined, carcinoma occurred in a 12 months old mouse, and 2 subcutaneous sarcomas in mice 11 and 12 months old.

It seems as if the irradiation product of 9,10-dimethyl-1,2-benzanthracene has lost one component of its carcinogenic power but retained another. Berenblum (3) supposes that "carcinogenesis is not a single process but consists of several component phases which may be dissociated." Possibly this irradiation product is a compound which may bring about what Berenblum calls preneoplastic hyperplasia, but is incapable of advancing the process further to manifest carcinoma as can the original hydrocarbon.

By oxidation of 9,10-dimethyl-1,2-benzanthracene with sodium bichromate ($Na_2Cr_2O_7$) the absorption spectrum is, as may be seen from C in Fig. 3, altered in quite another way than by irradiation. By painting with 9,10-dimethyl-1,2-benzanthracene oxi-

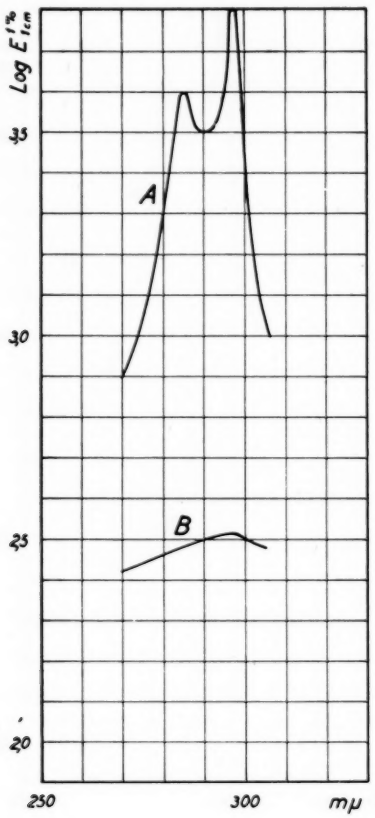

FIG. 1

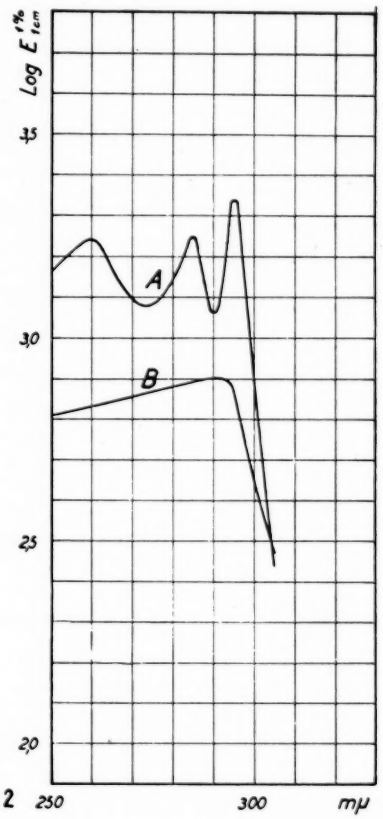

FIG. 2

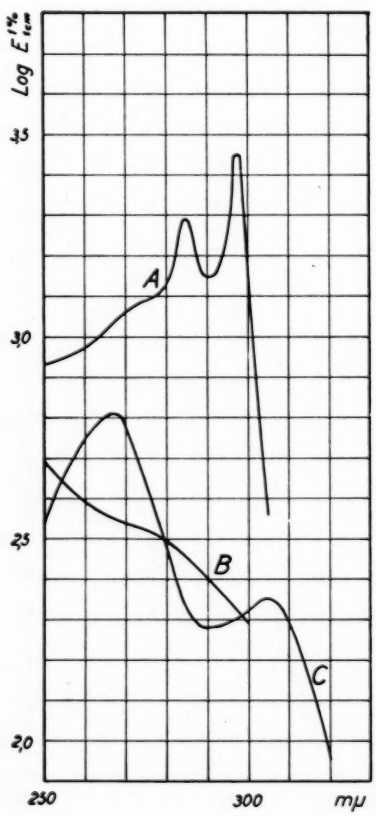

FIG. 3

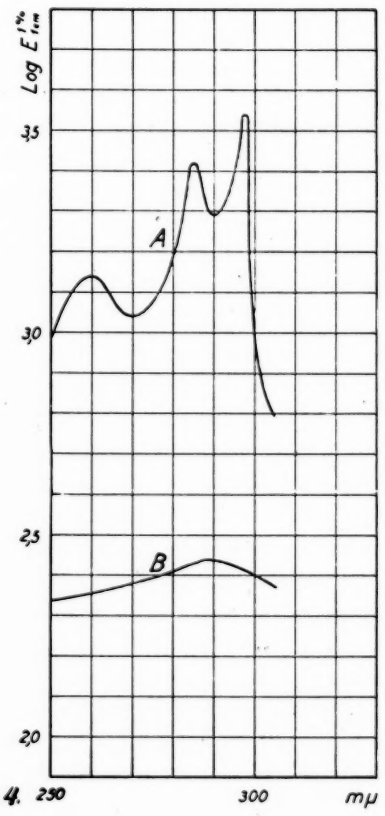

FIG. 4

dized with sodium bichromate no tumor development was seen among 30 animals 20 of which lived longer than 10 months. As mentioned above, in 1 animal a gastric ulcer was found. By oxidation with $Na_2Cr_2O_7$ the carcinogenic potency has thus been completely eliminated.

By subcutaneous injection of unaltered, irradiated, and sodium bichromate oxidized 9,10-dimethyl-1,2-benzanthracene, the following results were obtained:

Of 28 mice injected with 1 mgm. of original hydrocarbon, 10 sarcomas and 1 squamous cell carcinoma appeared in animals from 6 to 11 months old. In 2 of these, generalized leukemia and a large lymphosarcoma in the groin were found. The group of animals that did not develop sarcomas, produced 3 additional cases of leukemia, and 4 other animals developed small papillomas at the site of injection.

By injecting irradiated 9,10-dimethyl-1,2-benzanthracene into 18 animals only 1 sarcoma in a 12 month old mouse was produced. In 30 animals injected with the sodium bichromate oxidized hydrocarbon no tumors appeared at the site of application. One mouse 17 months old died of leukemia. In 7 animals small cysts (3 to 10 mm.) were seen; they contained a transparent liquid and their walls consisted of apparently normal connective tissue. Five of these cysts, which were left untouched, regressed spontaneously after several months.

By subcutaneous injection the carcinogenic potency of the irradiated hydrocarbon was thus reduced to a minimum and the sodium bichromate oxidized hydrocarbon was, as by painting, quite noncarcinogenic.

Methylcholanthrene.—The characteristic absorption spectrum of methylcholanthrene is totally changed after irradiation (Fig. 4). The hydrocarbon loses almost completely its absorption band in the ultraviolet region. The curves bear besides (Figs. 1 to 4), a great resemblance to the curves of 1,2,5,6-dibenzanthracene and its irradiation product. Both irradiation products, however, differ completely from a biological point of view since the irradiated 1,2,5,6-dibenzanthracene is less carcinogenic than the original hydrocarbon but nevertheless still has a distinct tumor-producing power; whereas the irradiation product of methylcholanthrene has for practical purposes lost its carcinogenic potency, as will be seen from the following:

By painting with the original hydrocarbon 13 cases of papillomas and 13 of carcinomas were produced in 27 mice. The tumors appeared rather quickly and none of the animals lived longer than 8 months. Of the 26 animals affected, 5 cases of leukemia and 1 of subcutaneous sarcoma at the place of painting were seen.

By painting with irradiated methylcholanthrene no tumor development at the place of painting was seen in 25 animals, but there was one case of leukemia in a 6 months old mouse.

By subcutaneous injection of 1 mgm. of original methylcholanthrene 25 sarcomas and 1 carcinoma appeared among 38 animals. Of the affected animals there was 1 case of leukemia and another of pulmonary adenocarcinoma and large gastric ulcer with carcinoma in addition to a sarcoma.

Of the animals subcutaneously injected with irradiated methylcholanthrene only 1 sarcoma occurred in the group of 20.

The carcinogenic potency of irradiated methylcholanthrene when injected is thus reduced to a minimum, and when applied by painting, is completely lost.

A summary of these results is shown in Table I. It demonstrates clearly the decreased carcinogenic potency of the irradiated hydrocarbons. Furthermore, another quality of the reduced carcinogenic potency of the irradiated hydrocarbon is seen, namely, that the irradiation mixtures have no or only slight accelerating effect on the characteristic spontaneous tumors, especially the leukemias, of mice of the Street strain. Among the animals treated by painting and by subcutaneous injection with original hydrocarbons a considerable increase in the incidence of leukemia is seen, and many of the leukemias appear at an earlier age than usual in this strain. Of 85 animals treated by painting with original hydrocarbons, 10 cases of leukemia were found, of which 3 were in the 9,10-dimethyl-1,2-benzanthracene-treated group and 5 in the methylcholanthrene-treated group. From 112 animals treated by subcutaneous injection of original hydrocarbons, 8 cases of leukemia occurred, 5 of which were in the 9,10-dimethyl-1,2-benzanthracene-treated group. Altogether,

DESCRIPTION OF FIGURES 1 THROUGH 4

FIG. 1.—A=Nonirradiated 1,2,5,6 dibenzanthracene. B=Irradiated 1,2,5,6-dibenzanthracene.

FIG. 2.—A=Nonirradiated 3,4-benzpyrene. B=Irradiated 3,4-benzpyrene.

FIG. 3.—A=Nonirradiated 9,10-dimethyl-1,2-benzanthracene. B=Irradiated 9,10-dimethyl-1,2-benzanthracene. C=Sodium bichromate oxidized 9,10-dimethyl-1,2-benzanthracene.

FIG. 4.—A=Nonirradiated methylcholanthrene. B=Irradiated methylcholanthrene.

TABLE I: SUMMARY OF RESULTS OF TEST OF CARCINOGENIC POTENCY OF IRRADIATED HYDROCARBON

A. SKIN PAINTING TWICE WEEKLY FOR 24 WEEKS

	1,2,5,6-Dibenzanthracene				3,4-Benzpyrene			
	Ratio Tumors to Mice	Kind of Tumors	Comments		Ratio Tumors to Mice	Kind of Tumors	Comments	
Original hydrocarbon	7/ /14+	Approx. 50%	6 carcinomas 1 papilloma 1 carcinoma	+1 case of breast cancer complicated by leukemia and 1 case of gastric ulcer with cancer.	20+/ /21	Approx. 100%	11+ carcinomas 9 papillomas	+1 case complicated by leukemia.
Irradiated hydrocarbon	5/ /20+	Approx. 25%	2 papillomas 2 sarcomas	+1 case of breast cancer	10/ /22	Approx. 50%	8 carcinomas 2 papillomas	

	9,10-Dimethyl-1,2-Benzanthracene				Methylcholanthrene			
	Ratio Tumors to Mice	Kind of Tumors	Comments		Ratio Tumors to Mice	Kind of Tumors	Comments	
Original hydrocarbon	12/ /23+	—	2 carcinomas 10 papillomas	+Violent toxic effect; all mice died before 5 months old. 3 cases of leukæmia and 1 carcinoma in salivary gland.	26+/ /27	Approx. 100%	13 carcinomas 13 papillomas	+5 cases of leukemia and 1 of subcutaneous sarcoma
Irradiated hydrocarbon	8+/ /22	—	1 carcinoma 2 sarcomas 5 papillomas	+Minimal papillomas, remained unaltered or regressed spontaneously; 4 cases of gastric ulcer, 2 complicated by carcinoma.	0/ /25+	0%	—	+1 case of leukemia
Hydrocarbon oxidized with $Na_2Cr_2O_7$	0/ /30+	0%	—	+1 case of gastric ulcer.	—		—	

B. SUBCUTANEOUS INJECTION

	1,2,5,6-Dibenzanthracene				3,4-Benzpyrene			
	Ratio Tumors to Mice	Kind of Tumors	Comments		Ratio Tumors to Mice	Kind of Tumors	Comments	
Original hydrocarbon	17+/ /26++	Approx. 65%	17 sarcomas	+1 case complicated by leukemia. ++1 case of breast cancer	7/ /20+	Approx. 33%	7 sarcomas	+1 case of leukemia and 1 pulmonary adenoma.
Irradiated hydrocarbon	4/ /27+	Approx. 15%	4 sarcomas	+2 cases of breast cancer, 1 of which complicated by palmonary adenoma, and 1 isolated case of case of pulmonary adenoma.	2/ /24+	Approx. 8%	2 sarcomas	+1 case of pulmonary carcinoma.

	9,10-Dimethyl-1,2-Benzanthracene				Methylcholanthrene			
	Ratio Tumors to Mice	Kind of Tumors	Comments		Ratio Tumors to Mice	Kind of Tumors	Comments	
Original hydrocarbon	11+/ /28++	Approx. 40%	10 sarcomas 1 carcinoma	+2 cases of leukemia and lymphosarcoma ++3 cases of leukemia	24+/ /38	Approx. 65%	23 sarcomas 1 carcinoma	+1 case of leukemia, and 1 of pulmonary carcinoma and gastric ulcer with carcinoma
Irradiated hydrocarbon	1/ /18	Approx. 5%	1 sarcoma		1/ /20	Approx. 5%	1 sarcoma	
Hydrocarbon oxidized with $Na_2Cr_2O_7$	0/ /30+	0%	—	+1 case of leukemia	—	—	—	—

from 197 animals treated with irradiated hydrocarbons only 1 case of leukemia was seen. Of 60 animals treated with 9,10-dimethyl-1,2-benzanthracene oxidized with sodium bichromate only 1 case of leukemia was found. The incidence of spontaneous leukemia in this strain is, as mentioned earlier, 1 to 2 per cent.

The figures in the individual groups are too small to estimate whether the irradiation products of the different hydrocarbons vary as regards the accelerating effect on the spontaneous development of leukemia of the strain. It is remarkable that most of the cases of leukemia appeared among the animals

No increase in pulmonary adenoma or carcinoma has been seen. Only 5 cases from all the animals have been found, but these tumors as a rule appear late in the life of the animals.

It has been mentioned that especially among the animals painted with irradiated 9,10-dimethyl-1,2-benzanthracene some cases of gastric ulcer were found and that these ulcers in some instances have been complicated by adenocarcinoma. Probably these lesions are not caused by the treatment since they have been found in untreated animals too. Street strain mice seems to develop gastric ulcers at an increasing rate.

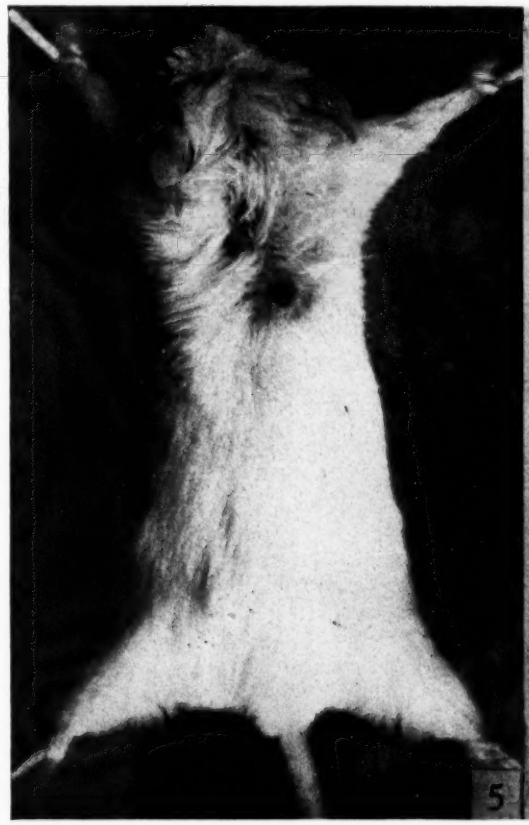

FIG. 5.—Mouse 46771, Street strain. Painted with photooxidized 9,10-dimethyl-1,2,benzanthracene. Small papillomas (1 carcinoma, see Fig. 6) developed after painting for 3 to 4 months; persisted unaltered for 7 months.

FIG. 6.—Mouse 46771, Street strain. Microphotograph of papilloma shown in Fig. 5. Condition seen to be squamous cell carcinoma. Mag. × 20.

treated with 9,10-dimethyl-1,2-benzanthracene, in spite of the fact that the life span of the animals in the painted group is so short.

It is remarkable also that another of the spontaneous tumors of the strain, mammary carcinoma, appeared only in the 1,2,5,6-dibenzanthracene group in 5 out of 70 females, while there have been no examples of the tumor in the other groups. This tumor appears spontaneously in the Street strain at the rate of about 7 per cent in non-breeding females.

CONCLUSIONS

The carcinogenic potency of the irradiation products of the carcinogenic hydrocarbon 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, 9,10-dimethyl-1,2-benzanthracene and methylcholanthrene, and of the product of oxidizing 9,10-dimethyl-1,2-benzanthracene with sodium bichromate ($Na_2Cr_2O_7$) has been investigated by means of painting and subcutaneous injection. The absorption spectra of the compounds used have been given. Irradiation effects a reduction

in the carcinogenic potency of these hydrocarbons but it has been impossible to find a correlation between the depression in the absorption intensity and the carcinogenic potency.

The irradiation products of 1,2,5,6-dibenzanthracene and 3,4-benzpyrene, which show different absorption curves, are both less carcinogenic than the original hydrocarbons, but the reduction of carcinogenic power is the same in the two hydrocarbons, namely, to approximately 25 per cent of the original hydrocarbon by subcutaneous injection and to approximately 50 per cent by painting. The irradiation product of methylcholanthrene, the absorption spectrum of which strikingly resembles that of irradiated 1,2,5,6-dibenzanthracene, has almost completely lost its carcinogenic potency.

9,10-Dimethyl-1,2-benzanthracene oxidized with $Na_2Cr_2O_7$ is quite noncarcinogenic, whereas the irradiation mixtures show greatly reduced carcinogenic potency. By painting with the irradiation product of 9,10-dimethyl-1,2-benzanthracene, many preneoplastic changes were seen, which in spite of continuous painting, in most cases remained at the same stage, or even regressed spontaneously.

The accelerating effect of the carcinogenic hydrocarbons on the spontaneous tumors in the strain used is reduced, although specific details cannot be deduced from the experiments.

It is probable that the results justify the suggestion that when an inhibitory effect has been noticed after concurrent application of ultraviolet light and carcinogenic hydrocarbons on the skin of mice this effect is due for the most part to photo-oxidation of the hydrocarbons.

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Spreading Factor in Human Carcinomas*

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The mechanisms responsible for the invasiveness of malignant tumors remain largely unknown. One explanation proposed is that malignant tumors may form a spreading factor that breaks down the barriers in surrounding normal tissues and thus permits penetration of tumor cells (6). If it exists, such a spreading factor would probably be hyaluronidase which hydrolyses the connective tissue constituent hyaluronic acid (12). Since previous studies (2,3,7) on hyaluronidase content of tumors have yielded indecisive results, and since improved methods are now available for assaying hyaluronidase, it seemed desirable to reinvestigate the problem. Specifically, experiments were designed to answer the question: is there evidence that human malignant tumors contain a spreading factor, such as hyaluronidase?

MATERIAL AND METHODS

The problem was investigated by making extracts of various carcinomas and of non-carcinomatous tissues, and by comparing their hyaluronidase content in 2 ways: first, by the extent of spread of a colored fluid in the skin of rabbits; second, by reduction in viscosity of hyaluronic acid solution.

Carcinomas, leiomyomas and normal organs were obtained after surgical removal or at autopsy, the tissues being refrigerated as soon as possible. Extracts were prepared according to the method of Chain and Duthie (4). From 2 to 30 gm. of tissue were weighed, minced, ground in a mortar with sand, frozen, and after thawing, extracted overnight in the refrigerator with usually twice the volume of M/10 sodium acetate adjusted to pH 4.6. The supernatant fluid was separated by centrifugation, and used without further purification.

Assay by spread in the skin.—Following the method, slightly modified, of Bacharach, Chance and Middleton (1), the back and flanks of rabbits were shaved, using only rabbits with unpigmented skin. The shaved area was marked off into 6 squares on each side of the midline, thus permitting 12 injections in each rabbit. As indicator, a saturated solution of hemoglobin in a balanced salt solution (Gey's solution) was used. Into each area was injected 0.2 cc. of this colored indicator mixed with 0.1 cc. tumor or tissue extract. Injections were made with a hypodermic needle and were intracutaneous.

Results were recorded 1 hour following each injection, by laying a piece of glass on the skin and tracing the usually round or oval outline of the area of diffusion. This procedure was immediately repeated twice. The outlines were subsequently measured with a planimeter and averaged. Usually 6 areas were injected with each test substance, and 6 control areas with Gey's solution. In the controls, the area of spread was about 0.5 sq. in.

Results of spread in skin.—Of 10 carcinoma extracts, 9 produced areas of diffusion larger on the average than control areas (Table I). Comparing each cancer extract with its control in Table I, it is computed (8) that differences of this magnitude or greater would occur by chance once or twice in 100 times. This justifies the conclusion

TABLE I: SPREAD OF HEMOGLOBIN SOLUTION IN RABBIT SKIN AS INFLUENCED BY EXTRACT OF HUMAN CARCINOMAS. AREA OF SPREAD 1 HOUR AFTER INJECTION

Site of carcinoma	Carcinoma extract, sq. in.	Saline control, sq. in.
Stomach	0.86	0.55
Lymph node	1.13	0.71
Thyroid	0.60	0.54
Breast	0.51	
Liver	0.58	
Thyroid gland	0.74	0.62
Sigmoid colon	1.05	
Rectum	0.72	0.59
Adrenal	0.65	
Colon	0.61	
Mean	0.74	0.60

* In all except one instance, the mean area of spread produced by extracts of carcinoma is greater than the mean control area. The combined means of the carcinoma series are significantly greater than the control series. These tumors were primary in the organ mentioned with the following exceptions: the carcinoma of lymph node originated in the stomach, that of the liver originated in the breast, that of the adrenal came from the lung.

*This investigatoin was aided by a grant from The Donner Foundation, Incorporated, Cancer Research Division.

that greater spread was caused by cancer extracts than by saline. However, the differences were great (50 per cent or more) in only 3 instances; hence the amount of spreading factor was apparently fairly large in 3 cancers, but not in the remaining 7.

When these results are compared with those produced by extracts of non-carcinomatous tissue, the contrast is distinct, for, as shown in Table II, extracts of normal organs and of benign tumors (leiomyomas) caused, on the average, no more spread than did saline. Only the adrenal appears to have contained a small but significant amount of spreading factor.

Lowering of viscosity of hyaluronic acid solution.—This is an approved method of assaying hyaluronidase (10,11). Hyaluronic acid was prepared from human umbilical cords (10) by extraction with water, followed by precipitation with alcohol and sodium acetate. The precipitate was washed and

TABLE II: SPREAD OF HEMOGLOBIN SOLUTION IN RABBIT SKIN AS INFLUENCED BY EXTRACT OF NON-CARCINOMATOUS HUMAN TISSUES. AREA OF SPREAD 1 HOUR AFTER INJECTION*

Tissue	Tissue extract, sq. in.	Saline control, sq. in.
Uterine fibroid	0.64	0.55
Uterine fibroid	0.66	0.71
Normal stomach	0.65	
Normal kidney	0.61	0.72
Normal liver	0.49	
Normal kidney	0.47	0.58
Normal colon	0.47	
Normal thyroid gland	0.49	0.50
Normal adrenal	0.64	
Mean	0.57	0.61

* Extracts of non-carcinomatous tissues produced on the average no greater spread than did salt solution.

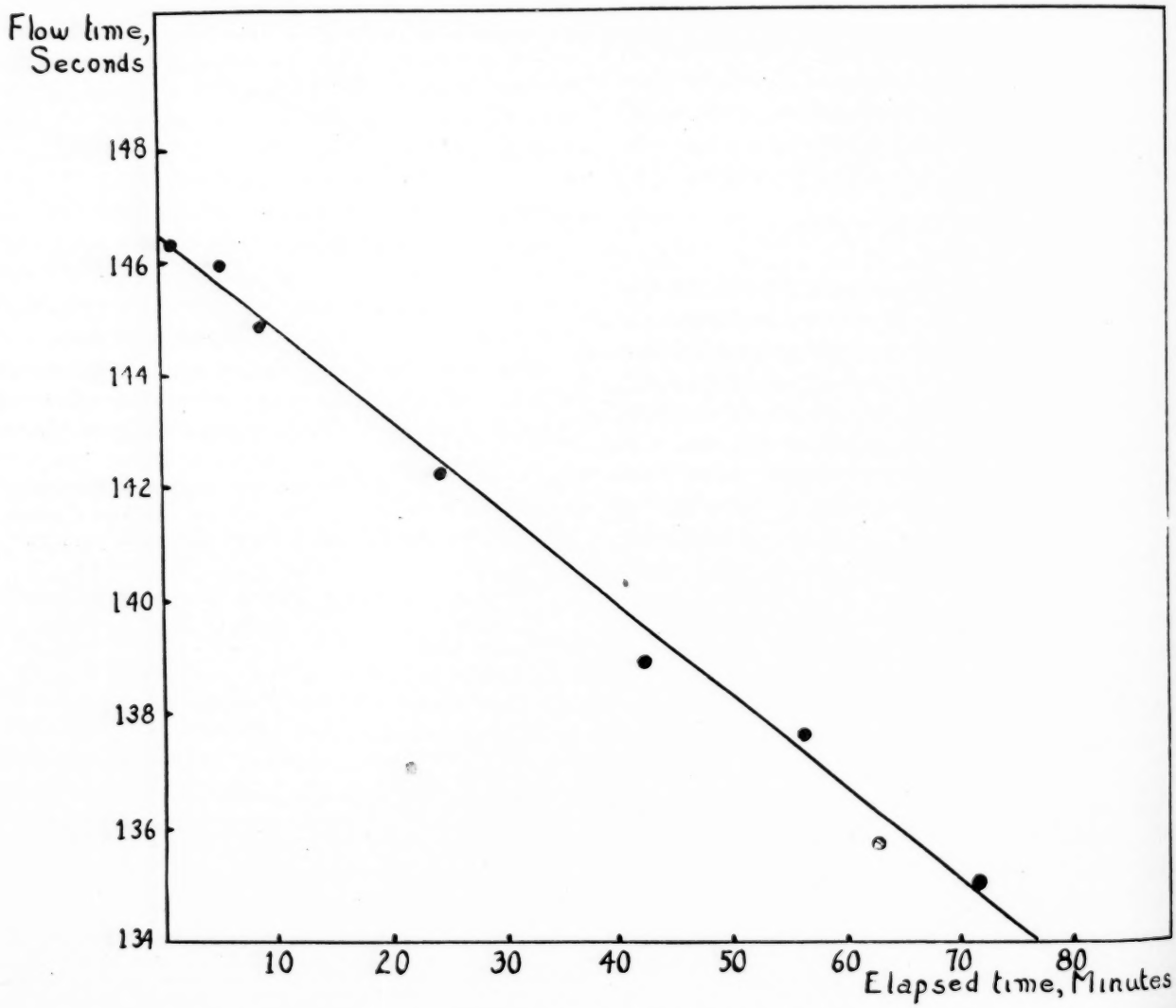

FIG. 1.—Results of viscometry with an extract of carcinoma of the breast and hyaluronic acid solution (from umbilical cord). Flow times have been plotted against times elapsed after mixing tissue extract with hyaluronic acid substrate. Graphs indicate slow, steady fall in viscosity of substrate.

dried. For viscosimetry, a 0.25 per cent solution of the dried and powdered preparation of umbilical cord was made up in molar NaCl. Four cc. of this solution were mixed with 1 cc. molar sodium acetate buffer of pH 4.8 and 1 cc. tissue extract, prepared as described above.

Decrease in viscosity was determined in the Ostwald viscosimeter, with which is measured the time required for liquid to flow through the viscosimeter tube by gravity. The initial flow in these experiments varied from 144 to 191 seconds at 30° C., the temperature being regulated by a water bath, with variations less than ±0.1° C. The flow time of the salt solution without tissue extract was about 80 sec.

In actual determinations, 2 viscosimeters were used simultaneously. In one the solution contained the tumor or tissue extract to be tested, while in the other, the solution contained tumor or tissue extract that had been heated at 100° C. for ½ hour, to inactivate any enzyme, and subsequently filtered. Thus, the second viscosimeter would show any chance contamination with a viscosity-lowering substance.

When a few grains of hyaluronidase[1] (prepared from bull's testis) were added to such a system, viscosity fell in about 10 min. to the value obtained with the salt solution alone. None of the extracts of tumors or organs, however, produced such rapid change in viscosity. Consequently an arbitrary measure of fall in viscosity was chosen, namely the number of seconds by which the flow time decreased during the first hour. The flow time at time zero was obtained by extrapolation.

TABLE III: DECREASE IN VISCOSITY OF HYALURONIC SOLUTION PRODUCED BY EXTRACTS OF CARCINOMAS*

Site of carcinoma	Initial flow time, sec.	Flow time after 1 hr., sec.	Decrease in flow time, sec.
Stomach	191	175	16
Lymph node	168	153	15
Thyroid gland	146	139	9
Breast	144	137	7
Liver	146	145†	—
Thyroid gland	154	135	19
Sigmoid colon	155	148	7

* With one exception, extracts of carcinoma caused decrease in viscosity of the substrate.
† After ½ hour.

Results of viscosimetry.— Of 7 carcinoma extracts tested, 6 lowered viscosity of umbilical cord extract, the flow time decreasing slowly but regularly (Table III and Fig. 1). Since the curve is far from its asymptote, it approximates a straight line.

In contrast to the carcinomas, extracts of non-carcinomatous tissues in general did not decrease flow time (Table IV). However, 2 extracts of normal kidneys lowered viscosity as much as did the carcinoma extracts. The explanation for this is obscure, as the kidneys contained no spreading factor as tested with rabbit skin.

TABLE IV: DECREASE IN VISCOSITY OF HYALURONIC ACID SOLUTION PRODUCED BY EXTRACTS OF NON-CARCINOMATOUS TISSUES*

Tissue	Initial flow time, sec.	Flow time after 1 hr., sec.	Decrease in flow time, sec.
Leiomyoma	193	193†	—
Leiomyoma	166	169†	—
Normal Liver	137	144†	—
Normal Stomach	144	143†	—
Normal Kidney	147	129	18
Normal Liver	148	152†	—
Normal Kidney	148	129	19

* Of non-carcinomatous extracts, only those of normal kidney caused decrease in viscosity of the substrate.
† After ½ hour.

DISCUSSION

It is concluded from these experiments that a spreading factor (probably hyaluronidase) is demonstrably present in some human carcinomas, but not in others. This conclusion is in agreement with those of other investigators. Thus, Duran-Reynals and Stewart (7) found a spreading factor in 11 of 28 human carcinomas, but in 13 sarcomas found none. Gibertini (9) reported that extracts of 5 of 23 human tumors strongly reduced viscosity of the substrate; there was no apparent difference in this respect between carcinomas and benign mesoblastic tumors. Working with transplantable animal tumors, mostly sarcomas, Boyland and McClean (3) found that extracts of many of them increased the spread through rabbit skin, and that their activity in this respect was correlated with growth rate. Also with animal tumors, Pirie (14) found hyaluronidase in some but not in all; his method of assay involved liberation of reducing sugars from a substrate believed to contain hyaluronic acid. Finally, Chain and Duthie (4) found no hyaluronidase in Jensen rat sarcoma (though Pirie had done so).

Thus it is evident that spreading factor can be demonstrated in some but not all malignant tumors. As compared with human testis, the concentration in tumors is low. Even so it is possible that a small amount of spreading factor, acting for a long time, may soften connective tissue enough to facilitate passage of tumor cells.

[1] Supplied through the kindness of Dr. Erwin Schwenk of the Schering Corp., Bloomfield, N. J.

That the spreading factor effective in the skin of rabbits and other animals is the same substance that reduces the viscosity of hyaluronic acid in a viscosimeter has been questioned (10), but this statement is regarded as probably true (11, 13). However, as stated above, extracts of normal kidneys were found to lower viscosity but not to enhance spread in the skin. This observation suggests the existence of some system other than hyaluronidase—hyaluronic acid; probably extract of umbilical cords contains other substances that may be hydrolyzed by appropriate enzymes.

Various normal organs were found by Claude and Duran-Reynals (5) to contain spreading factor in moderate amounts, whereas, Bergamini (2) found the majority of normal organs to possess no spreading factor, and only minimal amounts of viscosity-reducing substance.

SUMMARY AND CONCLUSIONS

The question was investigated whether human carcinomas contain a spreading factor such as hyaluronidase, which might be a factor in the mechanism of invasiveness. Extracts of a number of carcinomas, obtained either after surgical removal or at autopsy, were compared with extracts of leiomyomas and of normal organs.

The observations were made on the extent of spread of a colored solution (hemoglobin) caused by injection of the extract in the skin of the rabbit. Of 10 carcinomas, 9 produced areas of diffusion larger on the average than control areas (in controls, tissue extract was replaced by salt solution). However, in only 3 instances were the areas injected with carcinoma extract much larger (50% or more) than the controls. In contrast to carcinomas, extracts of leiomyomas and normal tissues did not, on the average, cause more spread than did saline solution.

The presence of hyaluronidase was tested by the ability of carcinoma and non-carcinoma extracts to lower the viscosity of hyaluronic acid obtained from umbilical cords. Extracts from 6 of 7 carcinomas caused slowly progressive decrease in viscosity, while of non-carcinomatous tissues, only 2 (extracts of normal kidney) decreased viscosity.

It is concluded from these experiments, as well as from others in the literature, that some cancers contain a spreading factor, though in low concentration. When present this spreading factor, probably hyaluronidase, may facilitate cancerous invasion of connective tissue.

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Failure of Hyaluronidase to Increase the Invasiveness of Neoplasms*

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In recent papers (1, 5) it was suggested that invasion of the adjacent normal tissues by malignant tumors may be facilitated by the presence of a spreading factor such as hyaluronidase. In support of this hypothesis, it was reported that some human carcinomas have been found to contain a spreading factor. Further support would be given to this hypothesis if it could be shown that invasiveness of neoplasms is increased by injection of hyaluronidase into or near a neoplasm, since the connective tissue barrier would presumably be weakened, and penetration of tumor cells facilitated. Accordingly, injections of hyaluronidase were made into tumors of two kinds, a transplantable mouse sarcoma and the Shope rabbit papilloma.

Experiments with a Transplantable Mouse Tumor

Mouse tumor 241 is a rapidly growing and metastasizing sarcoma, originally produced by a chemical carcinogen, transplantable in nearly 100 per cent of C57 black mice, and usually killing them in about 3 weeks. Since this tumor does not itself produce hyaluronidase,[1] any effect of injected hyaluronidase would not be masked.

Forty-eight mice were injected subcutaneously in the flank with tumor 241, and transplants became established in all animals. Subsequently twice a day, 0.25 mgm. of hyaluronidase in 0.25 cc. balanced salt solution was injected into or near the tumors in 24 mice, while the same number of control animals were injected with salt solution alone.

At the end of 19 days, many of the mice had succumbed and the rest, in poor condition, were sacrificed. At autopsy, all the tumors showed fixation to the skin and extension into the thoracic wall, without obvious differences between experimental and control animals. Microscopic sections of the lungs revealed metastases in 8 of the mice injected with hyaluronidase and in 6 of the controls.

In a second series of animals, 36 mice were injected in the flank with tumor 241, and subsequently 18 of the animals were injected 6 times a week with hyaluronidase, the remaining mice with salt solution alone.

Half the animals were sacrificed after 14 days, and the tumors were exposed in order to estimate fixation to the chest wall or other underlying structures. Of 9 animals injected with hyaluronidase, 8 showed fixation whereas in 9 animals injected with salt solution alone, the tumor was fixed in only 5. This difference is not statistically significant.[2]

The tumors were then dissected out and their volumes determined by measuring in a graduated cylinder the amount of liquid which they displaced. For tumors injected with hyaluronidase, the average volume was 2.2 cc.; for tumors injected with salt solution alone, 1.7 cc. The difference proved not to be statistically significant.[3]

A histologic section was prepared from the lungs of each mouse, and the metastatic tumors, if any, were counted. Of animals treated with hyaluronidase, 5 of 9 showed lung metastases and the total metastases were 7.[4] The metastatic growths ranged in size from 2 mm. in diameter to minute lesions. The control animals showed the same incidence of pulmonary metastasis, *i. e.*, 5 of 9 animals, and the total number of metastases was 6. It is concluded from these observations that significant differences

* This investigation was aided by a grant from The Donner Foundation, Incorporated, Cancer Research Division.

[1]As shown by failure of extract of this tumor to reduce the viscosity of hyaluronic acid (unpublished experiments) when tested by a method recently described (5).

[2]Differences of this magnitude or greater would be expected to occur by chance in more than 10 per cent but less than 20 per cent of such series.

[3]Equal or greater differences would occur by chance in 10 to 20 per cent of such series of experiments.

[4]As only one section was examined from each pair of lungs, it is likely that some minute metastases were missed.

in the 2 series of mice were not present 2 weeks after inoculation.

The remaining mice were sacrificed at intervals up to 24 days after inoculation. At autopsy, the tumors were adherent to deep structures in all except one animal in each series, and thus no difference in local invasiveness was evident. The average volume of the tumors was, for animals injected with hyaluronidase, 3.4 cc.; for control animals, 4.7 cc. All animals (9 in each series) had pulmonary metastases. The total number of pulmonary metastases in animals injected with hyaluronidase was 38; in control animals, 37. Metastases ranged in size from 4 mm. in diameter to less than 1 mm.

Thus from these experiments no evidence was obtained that repeated injection of hyaluronidase increased either local invasiveness or pulmonary metastasis.

Experiments with the Shope Rabbit Papilloma

In contrast to the invasive character of the mouse tumor used in the preceding experiments, the rabbit papilloma is essentially benign. That it can become invasive, however, has been demonstrated (7). Therefore, by injecting hyaluronidase into these tumors, it was hoped to increase the incidence and rate of invasiveness and malignant transformation, if hyaluronidase is capable of producing these effects.

Papillomas were induced by applying Shope virus to the dorsal skin of 29 rabbits of mixed breed, several drops of the virus preparation[5] being rubbed into a scarified area of about 1 sq. cm. In from 15 to 19 days, papillomas appeared in all the animals. Fifteen rabbits were subsequently injected every day in the tumor area with 1 cc. of balanced salt solution containing 0.5 mgm. of hyaluronidase.[6] The control group of 14 rabbits was injected with salt solution alone.

The experiment was terminated after 30 weeks, at which time 8 of the 15 rabbits which had received hyaluronidase showed complete regression of their papillomas, 6 had died of intercurrent infection between the first and 30th week with papillomas still present, and one survived with papilloma apparently regressing. When the rabbits whose papillomas had regressed were sacrificed, the sites of the tumors were no longer discernible and there were no metastases. In the 6 rabbits dying of intercurrent infection, autopsy revealed papillomas limited to the skin. There were no demonstrable metastases. Microscopically there was no invasion by the tumor of the underlying connective tissue.

Of the control series, in 5 rabbits the papillomas regressed and disappeared during the first 8 weeks, and the others were sacrificed or died at various times. None showed local invasiveness or metastatasis. From these experiments it is evident that injection of hyaluronidase into the rabbit papilloma did not cause invasiveness or metastasis nor otherwise apparently influence the growth of the tumors.

DISCUSSION

The two kinds of tumors selected for studying the effect of hyaluronidase on invasiveness differed greatly in their rate of growth, and in their tendency to invade locally and to metastasize, the mouse tumor 241 being highly malignant, the Shope rabbit papilloma usually benign but potentially malignant. It was hoped that one or the other grade of malignancy would be appropriate for demonstrating whether hyaluronidase could enhance invasiveness. That this proved not to be the case may indicate (a) that hyaluronidase has no power to increase invasiveness or (b) that appropriate tumors were not selected. A third explanation is possible, that is, that anti-hyaluronidase may have been formed as the result of repeated injection of hyaluronidase and that this inhibiting factor prevented any spreading effect or tendency of hyaluronidase to increase invasiveness (4).

The effect of spreading factor upon the rate of growth of tumors, rather than upon their invasiveness, has been studied by others. Duran-Reynals (2) reported inhibition of growth of the Brown-Pearce epitheloma of the rabbit when testicular extract was added to the tumor cell inoculum, but suggested the possibility that dilute testicular extract may act as a stimulant to tumor growth. Tanzer (8) found that of several transplantable mouse tumors some were inhibited by testicular extract while others were not affected. Similar results are reported for tumors in the rat, guinea pig, and rabbit by Prime and Haagensen (6). The subject has been reviewed recently by Duran-Reynals (3).

SUMMARY

Earlier experiments had suggested the possibility that invasiveness by malignant tumors may be facil-

[5] The virus preparation was made from glycerinated papillomas of cotton-tail rabbits obtained from Dr. J. W. Beard of Duke University. The papillomas were ground in a mortar with sand and extracted with 10 volumes of salt solution. The supernatant fluid, obtained by centrifugation, was used as the inoculum.

[6] Hyaluronidase was obtained through the courtesy of Dr. Erwin Schwenk of the Schering Corp., Bloomfield, N. J.

itated by the presence of a spreading factor such as hyaluronidase. It was thought that injection of this substance into or near a neoplasm might so alter the surrounding connective tissues as to permit more ready penetration by tumor cells.

This possibility was tested by repeated injection of hyaluronidase into: (a) 2 series of mice (C57 blacks) bearing tumor 241; and (b) into a series of rabbits bearing Shope papilloma. Injections were made into the tumors or their immediate vicinity. In each series control animals were injected with balanced salt solution alone.

All the mouse tumors grew rapidly and soon became locally invasive; many formed metastases in the lungs. There was no significant difference in local invasiveness, in size of tumors, or in number of pulmonary metastases between animals injected with hyaluronidase and those injected with saline alone.

In the papilloma-bearing rabbits, the tumors regressed in some, persisted in others, but in no instance gave evidence at autopsy of local invasiveness or metastasis. No difference was observed between rabbits injected with hyaluronidase and those receiving saline alone.

It is concluded that in these experiments, hyaluronidase did not promote local invasiveness or metastasis. Either hyaluronidase lacks the property of doing so, or the tumors selected were not appropriate, or anti-hyaluronidase may have been formed and prevented the expected results.

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Chemical Factors in the Mutual Adhesiveness of Epithelial Cells

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A method has recently been described for measuring the mutual adhesiveness of epithelial cells (3). It was found that cells of squamous cell carcinomas adhered to each other less strongly than did homologous normal epithelial cells. As the cause of this reduction in adhesiveness is not known, it seemed important to seek for an explanation of adhesiveness in normal cells, and to learn what changes would reduce their adhesiveness. If similar changes should be found in cancers, the reduced adhesiveness of their cells might be explained.

Until now, most of the work on cell adhesiveness has been limited to lower forms of animal life, and has been largely qualitative. In such studies it has been shown that calcium is essential for stability of the intercellular matrix (1). In calcium-free solution, blastomeres of marine eggs separate (8,12,13), and restoration of calcium to the fluid medium does not restore their adhesiveness (4). Adhesiveness of larval cells of sponges becomes reduced in calcium-free solution (11), and the most complete coalescence of dissociated sponge cells occurs only in the presence of calcium, magnesium and at least one univalent cation, sodium or potassium (5). The same cations maintain stability of the intercellular matrix in the gill cells of the mussel, *Mytilus edulis* (6); alkalies facilitate separation of these cells. Thus, in various lower forms of animal life, calcium and magnesium are important in maintaining adhesiveness of cells (14).

In view of investigations such as those cited above, experiments were designed to determine the effect of calcium and magnesium on adhesiveness of human epithelial cells. If lack of calcium lessened adhesiveness, the decreased adhesiveness observed in cancer might thereby be explained since cancer cells have been reported (2) to be deficient in calcium.

* This investigation was aided by a grant from The Donner Foundation, Incorporated, Cancer Research Division.

MATERIAL AND METHODS

As the method of measuring adhesiveness of cells has been described recently (3), only a summary will be given here. By means of micromanipulation, pairs of cells are separated from each other (Figs. 1 to 3), and the force required to do so is measured by the bend produced in a calibrated microneedle. The force required to separate the cells as expressed in milligrams is taken as a measure of adhesiveness. The cells, in the present experiment, were obtained from human buccal mucosa. All the experiments were done at room temperature, 20° C. approximately, and all measurements of cell adhesiveness were made after 5 to 30 minutes' immersion in the various test solutions.

In order to test the effects of various solutions, it was first necessary to determine the value of adhesiveness in balanced salt solution, this value then serving as a basis for comparison. Accordingly buccal epithelial cells were placed in Gey's solution[1] and 100 pairs of cells were separated. The average force required was 1.34 mgm. A comparison of this result with those obtained when the cells were subjected to various chemical alterations in environment is shown in Table I.

Effect of lack of calcium.—After 5 to 30 minutes immersion in a salt solution from which calcium was omitted, 50 pairs of cells were separated. It was found that the force required averaged 0.96 mgm. As shown in Table I, this value is significantly lower than that obtained for comparable cells in balanced salt solution, and it is concluded that mutual adhesiveness of epithelial cells is reduced in the absence of calcium.

Irreversibility of the effect caused by lack of calcium.—When cells were placed in calcium-free solution for 10 minutes, and adhesiveness was meas-

[1] Composition in grams:—NaCl - 8.0; KCl - 0.375 $CaCl_2$ - 0.275; $NaHCO_3$ - 0.25; $MgCl_2 \cdot 6H_2O$ - 0.21; Na_2HPO_4 - 0.12; KH_2PO_4 - 0.025; anhydrous dextrose 1.0; distilled water - 1 liter.

TABLE I: MEAN FORCES REQUIRED TO SEPARATE PAIRS OF CELLS BY MICROMANIPULATION, COMPARING VALUES OF ADHESIVENESS OBTAINED IN VARIOUS SOLUTIONS WITH THAT IN BALANCED SALT SOLUTION

Solution	Pairs of Cells	Mean and its Standard error, mgm.	Difference of means* Error of difference
Balanced saline	100	1.34 ± 0.056	
Calcium-free	50	0.96 ± 0.083	3.5
Magnesium-free	50	1.11 ± 0.066	2.7
Calcium and magnesium-free	50	0.62 ± 0.068	7.3
Potassium excess	35	1.25 ± 0.084	0.9
Methylcholanthrene	50	1.01 ± 0.072	3.6

* In the fourth column is given the ratio of the difference between the mean value of adhesiveness in balanced salt solution and the mean value in the test solution, divided by the standard error of the difference. When this ratio is equal to or greater than 2, it is regarded as significant.

TABLE II: MEAN FORCES REQUIRED TO SEPARATE PAIRS OF CELLS BY MICROMANIPULATION, COMPARING VALUES OF ADHESIVENESS OBTAINED IN VARIOUS SOLUTIONS WITH THAT IN CALCIUM-FREE SOLUTION

Solution	Pairs of Cells	Mean and its Standard error, mgm.	Difference of means* Error of difference
Calcium-free	50	0.96 ± 0.083	
Calcium-restored	35	1.04 ± 0.082	0.7
$[Co(NH_3)_6]Cl_3$ 4×10^{-4}M in Calcium-free	35	0.93 ± 0.067	—0.3
$[Co(NH_3)_6]Cl_3$ 1.2×10^{-3}M in Calcium-free	35	1.14 ± 0.104	1.4
Calcium and magnesium-free	50	0.62 ± 0.068	3.3

* See footnote to Table I.

ured, it was found to be of the same magnitude as that reported in the preceding paragraph. Other cells that had been in the calcium-free solution were now placed in balanced salt solution (containing calcium) for from 5 to 30 minutes, and the force required to separate 35 pairs of cells was determined. The average value was 1.04 mgm., which is not significantly greater than that in calcium-free solution (Table II). Thus under these conditions, reduction in adhesiveness brought about by lack of calcium in the medium was not reversed by restoration of calcium.

Failure of multivalent cations to restore adhesiveness.—In some physiological processes multivalent cations are more effective than bivalent cations such as calcium. For example, for maintainance of normal permeability in sea urchin eggs, a much smaller quantity of a multivalent cobaltamine is required than of calcium (10). It was therefore of interest to determine whether multivalent cobaltamines would restore normal adhesiveness to cells which had been exposed to a calcium-free solution. Accordingly the cobaltamine $[Co(NH_3)_6]Cl_3$, of which the cation is trivalent, was added in a concentration of 4×10^{-4} M [2] to a calcium-free salt solution, and its effect tested on 35 pairs of cells. To separate these cells requires a mean force of 0.93 mgm., which is not significantly different from the value obtained in calcium-free medium without cobalt (Table II).

Since the concentration of cobaltamine might have been too low, the experiment was repeated using 3 times as much. The mean value of adhesiveness was now 1.14 mgm., which, as shown in Table II, is not signficantly greater than that obtained in calcium-free solution without cobalt. Nor is this value signficantly less than that obtained in balanced salt solution. As this experiment is indecisive, further investigation will be necessary.

Lack of magnesium.—When cells were exposed to a solution, balanced except for absence of magnesium, the average value of adhesiveness was 1.11 mgm., which as compared with the value in balanced salt solution, is significantly less (Table I). Thus both calcium and magnesium are essential for maintaining normal adhesiveness.

Lack of both calcium and magnesium proved more effective in lowering adhesiveness than lack of either cation alone. When both these cations were omitted from the solution, the mean value of adhesiveness was 0.62 mgm., the lowest figure obtained in any solution tested (Table I and II), and significantly lower than in either calcium-free or magnesium-free solution.

Excess of potassium.—Since in some types of experiment potassium has an action antagonistic to that of calcium (7), adhesiveness was measured when potassium was present in excess. In a salt solution containing twice the normal amount of potassium (75 mgm. per 100 cc.), the mean force required to separate pairs of cells was 1.25 mgm., a value not significantly different from the control (Table I). Thus in the concentration used, excess of potassium had no effect.

Effect of methylcholanthrene on adhesiveness.—It must be emphasized that in the experiments reported above regarding the effect of reduced cal-

[2] Normal permeability of sea urchin eggs was maintained when the eggs were immersed in a calcium-free solution containing this concentration of cobaltamine (10).

cium on adhesiveness, calcium was withdrawn from the medium surrounding the cells, (and presumably from the cell surfaces), rather than from the cells directly. If it were possible to reduce the calcium content of the cells themselves, and if this procedure decreased adhesiveness, the result would strongly support the conclusion that lowered adhesiveness is dependent on reduced calcium content of cells.

Recently it has been shown that application of methylcholanthrene to the skin of mice causes decrease in calcium content of the epiderm, and causes further decrease when malignant epithelial growth appears as the result of the carcinogen (2). These experiments suggest that methylcholanthrene be tested for its effect on adhesiveness.

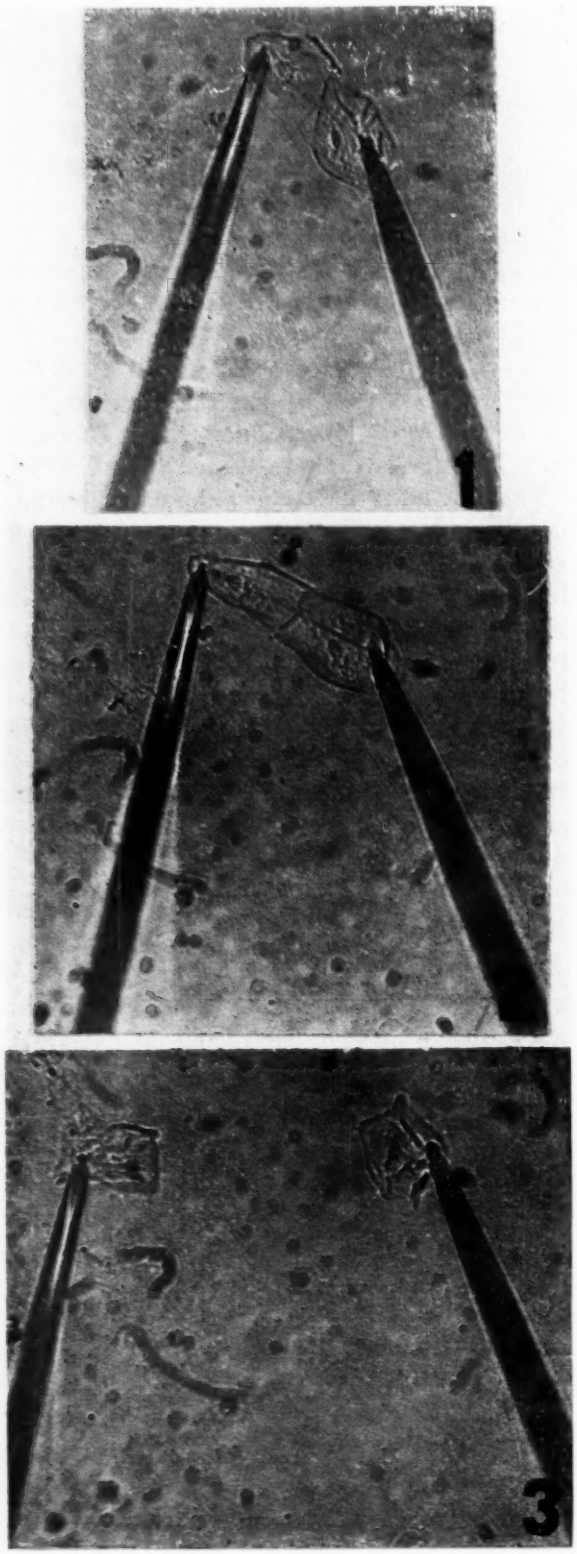
Accordingly a 0.6 per cent solution of methylcholanthrene in benzene was allowed to dry on a coverslip as a thin film on which the cells were then placed. The preparation was then immersed in balanced salt solution, and pairs of cells were separated by micromanipulation. The mean value of adhesiveness was 1.01 mgm., which is significantly lower than that of the control (Table I). Thus further evidence was obtained of a direct relationship between decreased calcium and lowered adhesiveness.

DISCUSSION

A concept of the action of calcium in maintaining adhesiveness of cells is presented by Höber (9) as follows: "The cells normally are packed together by a cementing substance with the character of a weak acid, forming rather insoluble Ca-salts, which, in the absence of Ca in the bathing salt solution, dissociate and are transformed into the softer and less resistant Na-salt." Höber goes on to say that these reactions take place in the intercellular space and at the cell surfaces. Thus the effects of calcium (and other bivalent cations) can be interpreted as dependent "upon more reversible or more irreversible reactions of these ions with the cementing substrata."

The experiments reported in the present paper, as well as those of other investigators mentioned in the introductory paragraph, are in accord with Höber's concept of the role of calcium and magnesium in maintaining normal adhesiveness.

Since cancer cells have been shown to be deficient in calcium, and since their mutual adhesiveness is reduced, a direct relation between these two phenomena is highly probable, and the conclusion seems



Progressive stages in process of cell separation by micromanipulation.

FIG. 1.—Microneedles have been inserted into pair of adherent epithelial cells.

FIG. 2.—One microneedle has been moved away from other, the cells are stretched and have developed tension lines in cytoplasm.

FIG. 3.—Cells after they have separated. Mag. × 154.

justified that local decrease in calcium is at least one major factor in lessening adhesiveness in cancer cells.

SUMMARY AND CONCLUSIONS

Experiments were designed to determine the chemical factors responsible for maintaining mutual adhesiveness of human squamous epithelial cells. The method used depended upon separation of pairs of cells by micromanipulation, the value of adhesiveness being determined by the degree of curvature produced in a calibrated microneedle when subjected to the strain required to detach the cells. It was found that:

1. Adhesiveness was decreased in the absence of calcium or magnesium, or both.

2. Decrease in adhesiveness brought about in a calcium-free solution was not reversed by restoring calcium to the medium.

3. Excess of potassium in the solution did not alter the adhesiveness.

4. Decrease in adhesiveness was produced by methylcholanthrene, a substance reported to lower the calcium content of squamous epithelium.

These results offer an explanation for changes in adhesiveness recently reported in malignant squamous epithelial cells. In these cancer cells, mutual adhesiveness was found decreased as compared with that of normal epithelium. Since the calcium content of cancer cells has been reported to be abnormally low, it seems highly probable that lessened adhesiveness of such cancer cells is partially explained by their deficiency in calcium.

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The Apparent Anticarcinogenic Action of Lanolin

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In the course of their work on the carcinogenic action of oils and tars on mouse's skin, Twort and Twort (4,5) observed that addition of anhydrous lanolin often led to a reduction in neoplastic response. The inhibitory effect was greater than that obtained with benzene as diluent, and also occurred when lanolin was applied separately to the skin during the intervals between paintings. In view of of this apparently specific inhibition of carcinogenesis, the use of lanolin was subsequently recommended as a preventive against occupational cancer in the mule-spinning industry (6).

A more pronounced inhibition of skin carcinogenesis by anhydrous lanolin was recently reported by Simpson, Carruthers and Cramer (1,2,3) in connection with methylcholanthrene. They found that a 0.3 per cent solution of this carcinogen in lanolin, applied thrice weekly for 14 weeks, was almost completely ineffective in inducing tumors or even in initiating those preliminary changes (epilation, destruction of sebaceous glands, epithelial hyperplasia etc.) which are regularly observed when the carcinogen is applied in benzene. This inhibitory effect was shown not to result from (a) inactivation of the carcinogen by the lanolin (b) the prevention of the carcinogen from reaching the epithelial cells, or (c) failure of the carcinogen to persist long enough *in situ*. Since the *amount* of methylcholanthrene applied was calculated to be adequate for carcinogenesis, and since the skin treated with the lanolin solution of the carcinogen, far from becoming resistant, was actually more responsive to subsequent painting with a solution of the carcinogen in benzene (3), they put forward the tentative hypothesis that "unaltered methylcholanthrene is a sensitizing agent, itself non-carcinogenic, that prepares the skin for subsequent action by metabolic derivatives of the carcinogen, or by substances formed in the tissues as a result of exposure to these metabolic products," with the implication that lanolin specifically interferes with this mechanism.

Irrespective of the possible merits of this hypothesis, acceptance of any specificity of action on the part of lanolin would be unjustified until simpler interpretations (based on such physical factors as a sub-threshold concentration of the carcinogen in the tissues) are definitely excluded.

When a benzene solution of methylcholanthrene is applied to the skin the benzene evaporates immediately leaving behind the carcinogen dissolved in the minute amounts of sebum normally present on the skin. The *effective* concentration of the carcinogen will therefore be much higher than the *original* concentration in the benzene. But when using a non-volatile solvent such as lanolin, the effective concentration practically corresponds to that of the solution as originally applied. It is hardly to be expected that two solutions of a carcinogen, one in a volatile and the other in a nonvolatile solvent, will elicit the same response, even though the *amount of* carcinogen applied and the *original concentration* are the same in both cases.[1]

Assuming that the inhibitory action of lanolin were entirely due to this dilution effect, one should expect to find (i) that with higher concentration, of the carcinogen in lanolin the neoplastic response should approximate that obtained in benzene, (ii) that other non-volatile solvents would behave similarly to lanolin, and (iii) that the effect should also be demonstrable with carcinogens other than methylcholanthrene.

The following experiments were undertaken to test these three possibilities.

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[1] A simple empirical confirmation of this was obtained by determining the relative concentrations of an irritant in a volatile and non-volatile solvent, respectively, required to produce comparable degrees of irritation in the mouse's skin. Using croton oil as an irritant, it was found that a 3.0 per cent solution in liquid paraffin produced approximately the same degree of change in the skin as a 0.5 per cent in acetone, thus indicating a six-fold increase in the *effective* concentration in the latter. The exact ratio will, of course, depend on the amount applied in the case of the volatile solvent (though not, presumably, in the case of the nonvolatile solvent.).

EXPERIMENTAL

The animals used for the work were white mice bred in this laboratory over a period of many years, representing a fairly homogeneous, though not genetically pure, strain. Twenty-five mice were used for each of the 9 experimental groups, applications being made to a small area of skin in the interscapular region, employing a capillary pipette for solutions in benzene, and a small glass spatula for the preparations in lanolin or liquid paraffin. Prior to each application, the hair was clipped with scissors, thus obviating the use of a chemical epilator.

The methylcholanthrene was made up as a 0.3 per cent solution in benzene, and as 0.3 per cent and 3.0 per cent solutions, respectively, in anhydrous lanolin (groups I-III, Table I). These were applied twice weekly until all the surviving animals had developed tumors after which the animals were left untreated until they died. The other carcinogen used in this investigation, 9,10-dimethyl-1,2-benzanthracene, being more potent than methylcholanthrene was applied only once weekly, in the following concentrations: 0.1 per cent in benzene, lanolin, and liquid paraffin, respectively, and 2.0 per cent in lanolin and liquid paraffin, respectively, treatment being continued until all the survivors had developed tumors. Careful note was made, at half-weekly intervals, of the survival rate in each group, and of the development of tumors (benign and malignant), malignancy being subsequently confirmed by histological examination.

Though the method used with methylcholanthrene was slightly different from that used by the previous workers (1,2), in that applications were made only twice weekly and continued beyond the 14th week, the results were essentially the same as theirs. Thus, the time taken for 50 per cent of survivors to develop tumors (hereafter refered to as "*the average response*") was 12½ weeks with 0.3 per cent in benzene, but 28 weeks with the same concentration in lanolin; while the total tumor yield was also greatly reduced in the latter (see groups I and II, Table I). However, with a 3.0 per cent solution in lanolin, the average response was, if anything, earlier than in the benzene control group (*i.e.* 10 weeks as compared with 12½ weeks), while the total tumor yield was at least as great as in the control group.

Similarly, with 9,10-dimethyl-1,2-benzanthracene the average response was delayed from 13 weeks, in the case of 0.1 per cent in benzene, to 23 weeks, in the case of 0.1 per cent in lanolin, with a commensurate reduction is total tumor yield. Once again, using a 1.0 per cent solution in lanolin, the average response was brought down to 12 weeks, whereas with a 2.0 per cent solution, it was as early as 8½ weeks with all survivors acquiring tumors in 13 weeks! (Compare groups IV to VII, Table I).

When this carcinogen was tested in liquid paraffin, the results were essentially the same as in lanolin, the average response being 23 weeks with 0.1 per cent, but 13½ weeks when a 1.0 per cent solution was employed. (Compare groups VIII and IX, Table I).

SUMMARY AND CONCLUSIONS

The striking diminution in the carcinogenic potency of methylcholanthrene, which occurs when lanolin is used as the diluent (1,2,3), has been confirmed, and found also to operate in connection with another carcinogen, 9,10-dimethyl-1,2-benzanthracene. In both cases, however, the inhibitory effect was completely nullified when the concentration of the carcinogen was raised. Furthermore, in the lower concentrations, an analogous diminution in carcinogenic potency was obtained by sub-

TABLE I: EFFECT OF CARCINOGENS IN VARIOUS SOLVENTS

Group	Carcinogen*	Concentrations, %	Solvent	Effective Total†	Tumor-bearing animals			First tumor, weeks	Tumors in 50% survivors, weeks	Tumors in 100% survivors, weeks
					Papilloma	Carcinoma	Total			
I	MC	0.3	benzene	16	9	**5**	14	7	**12½**	18
II	MC	0.3	lanolin	18	2	**1**	3	16	**28**	—
III	MC	3.0	"	17	6	**7**	13	6	**10**	15
IV	DMBA	0.1	benzene	25	10	**13**	23	6	**13**	24
V	DMBA	0.1	lanolin	19	9	**4**	13	13	**23**	31
VI	DMBA	1.0	"	24	9	**10**	19	4	**12**	18
VII	DMBA	2.0	"	18	8	**7**	15	4	**8½**	13
VIII	DMBA	0.1	liq. par.	16	8	**4**	12	12	**23**	37
IX	DMBA	1.0	"	23	8	**12**	20	7	**13½**	23

* MC=20-methylcholanthrene
DMBA=9:10-dimethyl-1:2-benzanthracene
† Number of survivors at the time of the appearance of the first papilloma in the group.

stituting liquid paraffin for lanolin as diluent.

These results are incompatible with the idea that the inhibitory effect of lanolin constitutes a specific anticarcinogenic action. They are readily accounted for, however, on grounds of inadequate concentration:

A 0.3 per cent solution of methylcholanthrene in benzene probably represents something approaching a 2.0 per cent effective concentration in the tissues,[1] since the benzene evaporates, leaving the carcinogen dissolved in the minute amounts of sebum on the skin. In the case of a solution in lanolin, the concentration remains unaltered and this (*i.e.* 0.3 per cent in the case of methylcholanthrene) is probably a subthreshold concentration for effective carcinogenesis.

The present results show that provided the concentration of the carcinogen is high enough, lanolin serves as an exceptionally favorable medium for facilitating carcinogenesis. In view of this, its recommended use as a preventive measure against occupational cancer (6) would seem to be of doubtful value.

Finally conclusions regarding the mechanism of carcinogenesis, based on the belief that the inhibitory effect of lanolin is specific (3), must be deemed invalid in the absence of more substantial support.

ACKNOWLEDGMENT

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Studies on the Relationship Between Sarcoma and Leukosis in Chickens

I. Tumor Induction by Intramuscular Injections of Cell-Free and Cell-Containing Material from a "Pure" Leukosis Strain

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It has been reported that some strains of transplantable fowl leukosis are capable of inducing sarcomas at the site of inoculation when suitable material is injected intramuscularly or subcutaneously. Observations on this subject have been published by Oberling and Guérin (18,19, 20), Rothe Meyer and Engelbreth-Holm, for strain E.S., (22, 6), Stubbs and Furth, for strain 13, (25). Histologically, the growths that arise with great regularity as a result of the inoculation of leukotic material are spindle cell and polymorph cell sarcomas; they may also present the aspects of endotheliomas or angioendotheliomas. The tumors develop fairly rapidly and may reach a considerable size. They can produce metastases and can be in most cases readily transplanted from fowl to fowl. The growths may also be induced by inoculation of cell-free leukotic material. The tumors are in most cases associated with leukosis, but may also appear alone. All such strains originated in chickens which had neoplasms in addition to some form of leukosis. It is generally assumed that in the case of the strains mentioned above the combined effect is caused by a single agent, originally capable, or becoming capable in the course of passage, of attacking not only primitive blood cells, but also other cell elements of the mesenchyme.

Besides these "combined" strains of leukosis, i.e. strains which are equally able to stimulate blood cell precursors and fixed mesenchymal cells to unrestricted growth, there are also leukotic strains more specifically adjusted. Thus, Stubbs (24) describes a strain of leukosis which never produced sarcomas after intramuscular or subcutaneous inoculations. Mohos' (17) erythroleukemic strain was also unable to produce sarcoma. Furth (7, 8) reported a strain of leukosis (strain 1) which after intramuscular inoculation led to sarcomas only in exceptional cases.[1] Uhl, Engelbreth-Holm and Rothe Meyer (27) studied two leukotic strains R. and T., which in contrast to their strain E.S., rarely led to tumor formation after intramuscular or subcutaneous inoculation. Strains of leukosis which never or relatively rarely cause new growth were designated "pure" strains.

Uhl, Engelbreth-Holm and Rothe Meyer (27) stressed the point that the tumors that occasionally arise after inoculation of material from their so-called "pure" strains of leukosis differ from those developing after the inoculation of "combined" strains not with respect to their frequency alone. Sarcomas which appear after the inoculation of "pure" strain material had a remarkably low growth capacity. Metastases were never observed. The transfer of such tumors was seldom successful and when feasible only one passage could be carried out.

From the reported observations it seems plain that different leukotic strains vary greatly in their ability to cause tumors, beginning with strains which have no tumor-producing capacity at all, and ending with those which constantly give rise to tumors as well as leukosis. It seems likely that in these extreme cases we are dealing on the one hand with an agent having special affinity for one definite type of cell (primitive blood cell) and this cell only, and on the other hand with an agent equally capable of attacking various cell elements of the mesenchyme.

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[1] It is possible, though not quite clear, that this strain has the same origin as that described by Stubbs (24).

No explanation has been offered for the peculiar behavior of so-called "pure" strains of leukosis that occupy an intermediate position in that, although they produce local growths, they do so irregularly, such growths being of very poor viability. Can the causative agent of these strains, like the agent of "combined" strains though to a lesser extent directly influence other cells (fibroblasts), in addition to those in which it is mainly effective (primitive blood cells)? Or is some other mechanism instrumental in the production of tumors after the inoculation of "pure" leukosis material? The purpose of the present paper was to provide information with respect to this question.

The experiments presented in this study were carried out with Engelbreth-Holm's strain T of hemocytoblastosis. In the first part of the investigation we demonstrate that the causative agent of hemocytoblastosis (strain T) has no tumor-producing power, and that local growths arise at the site of inoculation only when living leukotic blood cells (hemocytoblasts) are inoculated. In the second part we deal with the histology, histogenesis and transplantability of growths arising after the inoculation of leukotic cells and with the role played by the primitive blood cells in the origin of these growths.

FINDINGS

Experiment No. 1.—In this experiment a total of 20 chickens (white Leghorns), from 6 to 8 weeks of age, was used. Ten chickens were injected with whole blood or suspension of hemocytoblasts from leukotic fowls and the other 10 with cell-free leukotic plasma. The material was introduced deeply into the breast muscle of the recipients.

Blood for inoculations was withdrawn by means of heart puncture, when the diseased condition had fully developed and the blood contained overwhelming numbers of hemocytoblasts. Hemocytoblast suspensions were prepared by centrifuging citrated blood and suspending the buffy coat in a volume of Ringer's solution equal to that of the original blood. The plasma was obtained by centrifuging the citrated blood for 15 minutes at 3,000 rpm. It was further centrifuged 3 times for 15 minutes each time, before being injected; after each centrifugation only the upper two-thirds of the plasma were pipetted off with great care to prevent contamination with blood cells. Two-tenths to 1.0 ml. of plasma, whole blood or hemocytoblast suspension was injected into each fowl. The diagnosis was established in every instance by necropsy, blood smears and histological examination.

The results of the inoculations are recorded in Table I. The survival period of the inoculated birds varies from 8 to 55 days. All of the chickens inoculated with plasma developed leukosis. The inoculation of blood or hemocytoblast suspension caused leukosis in all but one case. In no case was plasma inoculation followed by the development of growths. In 6 out of 10 birds growth developed at the site of inoculation of the cell-containing material. The growths resulting at the site of inoculation of leukotic blood cells usually appeared 5 to 8 days after injection. Some of the growths were nodular and well circumscribed, the diameter of the nodules rarely exceeding 0.5 cm., while others were flat and spreading between the muscular bundles. They were often associated with hemorrhages.

TABLE I: RESULTS OF INTRAMUSCULAR INOCULATIONS OF LEUKOTIC BLOOD OR HEMOCYTOBLAST SUSPENSIONS (A), AND OF CELL-FREE LEUKOTIC PLASMA (B)

Chicken No.	Leukosis	Tumor	Length of life, days
	A		
2976	+	—	8
2983	+	—	11
2985	+	—	10
2987	—	+	46
3002	+	+	10
3027	+	—	8
3157*	+	+	8
3166*	+	+	16
3167*	+	+	12
2918	+	+	55
	B		
3068	+	—	12
3078	+	—	10
3140	+	—	37
3141	+	—	29
3142	+	—	36
3158	+	—	19
3162	+	—	12
3164	+	—	18
1438	+	—	28
1439	+	—	12

* Inoculation with hemocytoblast suspension.

Experiment No. 2.—There was a possible source of error in the design of the foregoing experiment. The concentration of the transmitting agent in blood cells and in plasma may not be commensurable and may be much higher in the cells than in the corresponding volume of plasma. The observed difference between the tumor-producing ability of cell-free and cell-containing material might thus be attributed to this factor. Another procedure for separating the causative agent from the viable cells was, therefore, adopted in order to make the tests as comparable as possible.

It has been established that a wide gap exists between the dosage of x-rays necessary for inactivation of the causative agent of fowl tumors and fowl leukosis and those that prevent a further multiplication of the cells. Five thousand r units suffice entirely to suppress *in vitro* the ability of normal and malignant cells to multiply (2,3,12) and doses up to 6,000 r units when applied to tumor fragments previous to inoculation invariably prevent the development of tumors in experimental animals (1,9-11,13,15, 16,21,23,26). On the other hand, many millions of r units are required for complete inactivation of the leukotic agent (4). The use of x-rays seemed to us to afford an excellent method for the investigation of the problem in question, because in this manner the material may be freed of viable cells with absolute certainty, while, at the same time, the causative agent is safeguarded in practically its original concentration.

Irradiations were carried out with a demountable x-ray tube working at 35 kv. on a current of 15 ma. The tube had a copper anticathode and a window of aluminum foil 30 mμ in thickness. Absorption analysis showed that the rays penetrating through the window foil and the 0.03 mm. mica coverslip were mainly the characteristic K-rays. Radiation was administered at a target-object distance of 3.7 cm.; the x-ray intensity at the distance of the irradiated object was about 80,000 r/min. for a tube current of 15 ma.

We selected liver as experimental material, the capillaries of leukotic liver being always engorged with primitive blood cells. The data of the exploratory experiments presented in Table II show that the inoculation of leukotic liver was in all cases followed by the development of tumors at the site of inoculation in addition to leukosis.

TABLE II: RESULTS OF INTRAMUSCULAR INOCULATION WITH FRAGMENTS OF LEUKOTIC LIVERS

Chicken No.	Leukosis	Tumor	Length of life, days
3400	+	+	13
3401	+	+	16
3407 right	+	+	13
" left		+	
3408 right	+	+	12
" left		+	
3413 right	+	+	19
" left		+	
3414 right	+	+	11
" left		+	

The design of the experiment was as follows: The liver of the leukotic fowl was cut into pieces of approximately 0.5 mm. in diameter. Ten fragments were placed in a depression slide covered with a mica cover slip and sealed with paraffin. The pieces of liver were x-rayed with a dose of 50,000 r units, this being 10 times the dose necessary for completely arresting mitosis. Non-irradiated pieces treated in the same way served as controls.

Inoculations were carried out by implanting the fragments of the liver into the pectoral muscle of the recipient. Each bird received 2 inoculations: the nonirradiated liver fragment at one side of the sternal keel and the x-rayed fragment at the other.

The results of the experiment appear in Table III. The survival time of the chickens successfully inoculated varies from 11 to 31 days. From a total of 16 chickens inoculated, 3 developed neither leukosis nor tumors after 40, 44 and 60 days respectively, at which time they succumbed to intercurrent diseases. Of the 13 remaining chickens all but one developed leukosis. The inoculation of irradiated material in no case resulted in growth at the site of inoculation, whereas the inoculation of nonirradiated liver pieces was followed by tumor formation at the site of inoculation in 12 of the 13 cases.

The tumors arising at the site of inoculation of leukotic livers have the same character as those which appear following the inoculation of leukotic blood cells.

TABLE III: RESULTS OF INOCULATION WITH X-RAYED AND NONIRRADIATED LEUKOTIC LIVER FRAGMENTS

Chicken No.	Leukosis	Tumors at the site of inoculation of: X-Rayed Material	Tumors at the site of inoculation of: Nonirradiated Material	Length of life, days
3262	+	—	+	15
3263	+	—	+	14
3271	+	—	+	31
3272	—	—	—	40*
3273	+	—	+	14
3274	+	—	+	15
3292	—	—	—	44*
3293	+	—	+	16
3331	—	—	+	32†
3332	+	—	+	14
3333	—	—	—	60*
3334	+	—	+	19
3343	+	—	+	13
3344	+	—	—	13
3352	+	—	+	11
3353	+	—	+	11

* Succumbed to intercurrent disease.
† Tumor regressed.

DISCUSSION

The results of the present study confirm and amplify the previous observations (14, 27) that the intramuscular inoculation of blood or organ fragments deriving from the so-called "pure" leukosis

strain leads to the development of growths at the site of inoculation in addition to hemacytoblastosis. In our experiments the percentage of tumor takes after the intramuscular inoculation of cell-containing leukotic material (strain T of Engelbreth-Holm) proved very high.

The data presented appear, furthermore, to provide affirmative evidence that the presence of viable primitive blood cells is definitely necessary for the formation of tumors by material from a "pure" leukosis strain. Out of a total of 26 birds inoculated with material free from viable cells (cell-free plasma, irradiated livers) but containing the transmitting agent of leukosis, none developed sarcomas at the site of the inoculation, whereas the cell-containing material gave rise to tumors following 22 out of 26 inoculations of leukotic livers as well as in 6 out of 10 birds inoculated with whole blood or hemocytoblast suspensions.

The fact that there is a noticeable difference between "combined" and "pure" strains of leukosis with respect to the number of tumor takes depending on whether the inoculum does or does not contain cells has already been stressed by Uhl, Engelbreth-Holm and Rothe Meyer (27). They observed that after injection of cell-containing material a far greater number of sarcomas developed than following administration of cell-free plasma. But the injections of cell-free plasma had also given rise to growths, although in a small number of cases. The authors concluded, therefore, that the agent of "pure" leukosis, as such, has tumor-producing properties.

In this point the results of our experiments deviate from those of Uhl, Engelbreth-Holm and Rothe Meyer. Our data suggest that the difference between inoculations of cell-free and cell-containing material in the case of "pure" strain (Engelbreth-Holm) is qualitative rather than quantitative, since we found that tumors were never caused by the injection of material free from viable cells. This discrepancy may possibly be due to differences in the technic of obtaining cell-free material. Whereas we took particular precautions in obtaining plasma free from leukotic cells, Uhl, Engelbreth-Holm and Rothe Meyer make no mention of any special measures taken to prevent contamination with blood cells, and such contamination may readily occur. An alternative possibility is a change in the properties of the transmitting agent of the leukosis strain, which it may have undergone during the course of passages over a period of many years.

The gross and microscopic pathology, histogenesis, and transmissibility of growths arising as a result of inoculation of leukotic blood cells will be reported in the following parts of this series.

SUMMARY

The tumor-producing properties of a so-called "pure" leukosis strain (hemacytoblastosis, strain I of Engelbreth-Holm) were investigated.

It was found that the intramuscular injection of (a) plasma from a leukotic fowl, free from cells but containing the transmitting agent, and of (b) livers from a leukotic fowl freed from viable hemocytoblasts by previous x-ray treatment, was not followed by the development of local growths. Inocula of the whole blood and nonirradiated liver fragments containing viable leukotic cells induced growths at the site of inoculation in a high percentage of cases.

The experiments indicate that growths arising after the inoculation of cell-containing leukotic material are not due to the direct action of the leukotic agent on connective tissue cells but to some other processes connected with the presence of viable hemocytoblasts in the inoculated material.

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Chemotherapy Investigations in Cancer

With Reference to the Influence of Certain Organic Dibasic Acids, Diamino Compounds and Nitro Compounds on Tumors in Mice

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Although knowledge of many aspects of cancer, including the chemistry of the cell, is rapidly expanding, it is still impossible to hazard any but very tentative hypotheses for guidance in selecting compounds for testing possible therapeutic value against tumors. In the past, substances have been investigated, in the majority of cases, on an empirical basis. A reasonable approach would be to observe the properties of members of various classes of compounds. It might be possible in this way to eliminate many categories and obtain information indicating what types are likely to form a basis for yielding effective material.

Therefore, though aware that in the absence of sound guiding principles such experiments can be explorative only in nature, we have carried out tests on various groups of compounds from this point of view. Some of these which may be of interest to other workers are briefly described in this contribution which deals with the action of some substituted succinic acids, a number of amino complexes and certain nitro compounds on three types of mouse tumor.

Boyland (2), by feeding certain dibasic acids, obtained evidence of distinct inhibitory effects on mouse tumors, one of the most active being malonic acid. The series of substituted succinic acids were made available by Mr. Garfield Thomas (biochemist to Queen Elizabeth Hospital, Birmingham). The amino compounds were supplied chiefly by Messrs. May and Baker and in some instances are among the substances investigated by Boyland (3) who, however, employed different tumors and technics. They all possess trypanocidal properties. Complexes with several of the amino compounds with nucleic acids were also tested, for some of which I am indebted to Prof. M. Stacey, Chemistry Department, University of Birmingham.

TECHNICAL METHODS

A. *Tumors and animals used.*—The tumors used for these experiments were:

I. Growths produced on mice by repeated applications of benzpyrene in acetone to the skin. The tests were commenced when the warts had been visible for several weeks and had an appearance suggesting epithelioma. Such tumors rarely regress even in the mixed strain of mice employed for inducing the growths.

II. Transplants from a spindle-celled sarcoma originally induced with dibenzanthracene in this laboratory and previously described (8). An inbred but unnamed strain of mouse was used, in the leg muscle of which this tumor grows regularly. The tumors are suitable for tests about 7 days after inoculation. The transplants utilized were those after some 140 or more passages.

III. Transplants of "carcinoma 63" which had been through about 20 passages in this laboratory when these tests were commenced.

B. *Injections.*—In the experiments described here injections were chiefly subcutaneous or intraperitoneal, at a distance from the tumor, twice or three times each week. Intravenous injections have been given where the reaction suggested that the limited number of injections possible by this route might be effective. There is evidence that some substances, quite ineffective by other routes, are inhibitory or even curative against certain mouse tumors when injected intravenously (11). The various organic acids were easily soluble as such, or a soluble sodium salt could easily be made, and they proved quite without toxic effects when injected into control animals in relatively large amounts. When the more poisonous substances, e.g. the nitrated series, were tested, the lethal dose was determined and doses of one-fifth to one-tenth lethal were given in a volume of 0.2 ml. for the subcutaneous injections and 0.10 ml. for the intravenous ones. When the substance was not soluble enough to afford a suitable aqueous solution a colloidal suspension stabilized by a small amount of gum or gelatine, was employed.

The correlation of numerical designations with chemical compounds is as follows:

654	4,4′-diaminostilbene
736	4,4′-diamidinodiphenylether
688	4,4′-diaminodiphenylether
875	4,4′-diamidinodiphenylurea diacetate
800	4,4′-diamidino-1,5-dephenoxypentane dihydrochloride
971	4,4′-diaminoazobenzene
952	Disulphanilamide
967	4,4′-diaminodiphenylthiourea

C. *Control tests.*—It has been shown that many experiments designed to demonstrate the inhibitory power of chemicals on the growths of animal tumors have been invalidated by disregard for the concomitant effect on the growth rate of the host and that apparent inhibition must be reviewed in relation to general toxic properties. Thus the tumor-inhibiting action of carcinogenic hydrocarbons given subcutaneously, appears to be largely incidental to the general disturbance of the metabolism of the animal (11). In order to control inhibition tests adequately, therefore, two control series are required, one in which the weight and health of noncancerous mice receiving similar injections are observed, and another of tumor-bearing mice which are allowed to continue without treatment.

An estimate of the effects of treatment of the skin epitheliomas was made by comparing the size of the tumors at the base before and after the injections. In the experiments with "Carcinoma 63" the volumes of the nodules were computed, while for the sarcomas, since it was difficult to get a satisfac-

TABLE I: TESTS WITH DERIVATIVES OF DIBASIC ACIDS ON MICE BEARING TRANSPLANTED 'DIBENZANTHRACENE' SARCOMAS. (136TH-145TH GRAFTS.) 7TH-9TH DAY AFTER INOCULATION. SUBCUTANEOUS INJECTIONS OF 2 MGM.

Group	Treatment	No. of animals	No. of injections	Final av. wt. of tumors, gm.	Initial av. wt. of animals, gm.	Final av. wt. of animals, gm.	Result
1	Succinic acid	5	10	3.7	30	32	No inhibition
2	Methyl succinic acid	10	11	2.85	33	35	Some inhibition
3	Phenyl succinic acid	8	8	3.85	29	32	No inhibition
4	Controls to 10 Gps. 1, 2, 3,	10	—	3.65	32	35	—
5	Cyclohexyl succinic acid	8	8	2.7	28.8	33	Some inhibition
6	Asymdimethyl succinic acid	10	9	3.63	32	35	No inhibition
7	Cyclohexyl-1-acetyl-1-carboxyl succinic acid	10	10	2.72	35	38	Some inhibition
8	Controls to 4, 5, 6	10	—	3.8	31	33	—
9	1-Hydroxyl-1-carboxyl- cyclo-hexane	9	10	3.8	30.6	30	No inhibition
10	Malonic acid	6	10	3.0	30	32	Some inhibition
11	Glutaric acid	6	10	3.9	34	36	No inhibition
12	Controls to 9, 10, 11	10	10	3.8	34	37	—

TABLE II: TESTS WITH COMPLEXES ON MICE WITH TRANSPLANTED DIBENZANTHRACENE SARCOMA (180TH-190TH TRANSPLANTS). SUBCUTANEOUS INJECTIONS 0.20 ML. = 0.5 MGM.

Group	Treatment	No. of animals	No. of injections	Av. initial wt. of animals, gm.	Av. final wt. of animals gm.	Av. wts. of tumors gm.	Result
1	654	6	8	32	34	3.7	No inhibition
2	736	6	8	32	32	3.8	No inhibition
3	Ribonucleic acid complex with 736	7	7	30.6	32	2.4	Definite inhibition
4	688	5	8	31	33.5	3.5	No inhibition
5	Ribonucleic acid complex with 688	6	7	30.6	32	2.6	Definite inhibition
6	967	6	9	28.4	29.5	2.1	Definite inhibition
7	875	6	8	30.6	32	3.5	No inhibition
8	Desoxyribonucleic acid complex with 875	6	8	25.6	30.5	3.6	No inhibition
9	Desoxynucleic acid complex with 688	7	7	35	36.5	2.7	Definite inhibition
10	800	6	7	33	34	3.8	No inhibition
11	971	6	8	28	29.5	3.4	No inhibition
12	952	6	7	30	33	3.75	No inhibition
13	Prontosil R	5	8	25.3	27	3.5	No inhibition

Average tumor weight in 4 tumor control groups = 3.75 gm.

tory value for the initial size, the final weight of the tumors after removing normal tissue was compared in the injected and control series.

RESULTS

Data for the tests with the dibasic acids and amino compounds on the sarcoma are given in Table I and II. Full details are not given for the other experiments since most of the substances produced only slight or no inhibition.

When tested by intravenous injections (2 mgm. in 0.10 ml.) on the transplanted dibenzanthracene sarcoma (150th to 160th grafts), commencing 7 days after implantation and administered in 5 to 6 injections in a period of 3 weeks, the following acid compounds gave no evidence of inhibitory action:

Succinic acid, phenyl succinic acid, asymetric dimethyl succinic acid, 1-hydroxyl, 1-carboxyl cyclo hexane, glutaric acid.

The following were somewhat inhibitory as indicated by the average final weights of the tumors compared with those of control animals. The health of the animals was not impaired by the injections.

Methyl succinic acid (Tumor wt. = 2.41 gm.)
Cyclohexyl succinic acid (tumor wt. = 2.41 gm.)
Cyclohexyl,1-acetyl,1-carboxylic (tumor wt. = 2.74 gm.)
Malonic acid ..... (tumor wt. = 3.0 gm.)
Controls ..... (tumor wt. = 3.4 gm.)

Methyl succinic acid, cyclo-hexyl succinic acid and cyclo hexyl,1-acetyl,1-carboxylic acid, also inhibited the growth of the benzpyrene-induced papillomas. The ratios of average final size to initial size of the tumors in groups of 5 to 8 animals injected subcutaneously for 5 weeks (10 to 12 injections of 2 mgm.) were:

Methyl succinic	14
Cyclohexyl succinic	13
Cyclohexyl-1-acetyl-1-carboxyl	14
Controls	19

The two latter compounds were also inhibitory towards carcinoma 63 but methyl succinic acid was inert as were the other compounds of this series tested.

In the experiments with this series of compounds injected subcutaneously into animals bearing carcinoma 63, the following compounds proved to be slightly inhibitory, the others being quite inactive. The ratios for the average final size of the tumors compared with the average initial size is given for the several groups of active substances.

Compound	Ratio
No. 736	9.0
Ribonucleic acid complex with 736	6.8
No. 688	9.4
Desoxyribonucleic acid complex with 688	8.8
Ribonucleic acid complex with 688	6.3
No. 971	9.0
Controls	10.2

The series of nitro compounds gave the following results:

Nitrovanillin (and vanillin) 2,4-dinitrophenol, nitroguanidine, and fluorene were inert when tested either on the dibenzanthracene tumors or on carcinoma 63. Some inhibitory action was demonstrated against either tumor by 2-nitrofluorene.

DISCUSSION

The majority of the substances tested showed no inhibitory action on the tumors by either method of injection, and none caused entire regression of any tumor. The distinct inhibitory action of malonic acid, which contrasts with the inactivity of succinic acid and glutaric acid, confirms the findings of Boyland (2) who observed 3 to 4 times the inhibition by malonic compared with glutaric by feeding mice bearing grafted sarcomas or spontaneous carinomas. The effect does not appear to be due to any action on the general condition of the mice, which maintained or increased their weight during the experiment.

It is of interest that 3 other compounds in this group also had inhibitory action at least as effective as malonic acid, including the dibasic acids containing the cyclohexyl structure.

This is not retained when the structure is altered so that only one carboxyl group remains. The general properties were similar for each type of tumor with the exception that methyl succinic acid did not appear to be effective against carcinoma 63. Crabtree, (5) who found that malonic acid did not delay the production of skin tumors in mice painted with a solution of 3,4-benpyrene, states that it does not combine with SH groups, having been used in biochemical studies as an inhibitor of succinic dehydrogenase, towards which it functions as a competitive substrate with effectiveness depending on the ratio of its concentration to that of succinic acid. It might be of interest to test whether this series of acids might act in a somewhat analogous way towards the enzyme.

On examining the results with the amino compounds there are 2 points of interest. Firstly, our sarcoma was somewhat less responsive to the action of the chemicals than the carcinoma and, also probably, than the tumor utilized by Boyland (3). Thus with his technic those compounds which gave an inhibition of 8 to 20 per cent had little effect on our

sarcoma, while the more effective compound 967 (4′4′-diaminodiphenylthiourea) produced some inhibition on this strain. Secondly, the 2 complexes with ribonucleic acid gave evidence of definite inhibitory power. The desoxyribo complex with 688 was also active in a lesser degree. In view of the small number of compounds tested it would be unwise to speculate on what part the ribonucleic acid may play in such action. It may be pointed out, however, that Parsons (9,10) has shown that the nucleotides adenylic acid and guanylic acid produce changes in the blood and tissues of normal and tumor-bearing mice and an inhibitory action on mouse tumors. Bertolotto (1) asserted that a purified "nucleoprotein B" isolated from the same type of tumor (Ehrlich adenocarcinoma) caused inhibition of tumor growth.

Among the nitro derivatives the activity of nitrofluorene was sufficient to classify it as somewhat inhibitory towards the sarcoma. Nitro compounds, particularly dinitrophenol and dinitrocresol, have the property of accelerating metabolism. The effect of dinitrophenol on white rats by feeding and injection was tested by Emge, Wulff and Tainter (6), who found no action on the growth rate of implanted tumor but an increase in their vascularity and certain cellular changes. Bischoff and Long (4) also found no significant change in the tumors of mice (Sarcoma 180) after feeding or injection of dinitrophenol. Kreyberg (7) observed earlier production of skin tumors and a greater number of tumors in mice painted with coal tar in the series receiving 0.10 to 2 mgm. dinitrocresol daily in food.

SUMMARY

The effects of subcutaneous and intravenous injections of a series of substituted succinic acids have been observed on the growth of a dibenzanthracene-induced, transplanted mouse sarcoma, and of subcutaneous injections on benzpyrene-induced papillomas and on carcinoma 63.

The effects of subcutaneous injections of a series of amino compounds, and a number of nucleic acid complexes with these, have been observed on mice with carcinoma 63 and dibenzanthracene-induced sarcomas, and the effects of subcutaneous injections of some nitro organic compounds on these tumors have also been studied. The experiments were controlled by "weight control" animals and "tumor controls". None of these substances caused complete regression of any of the tumors but a definite inhibitory effect of the following was observed:

Methyl succinic acid, cyclohexyl succinic acid, cyclohexyl acetyl-1-carboxylic acid, malonic acid, 4′4′-diaminodiphenylthiourea, 4,4′-diaminoazobenzene, 4,4′-diaminodiphenylether complex with ribonucleic acid, 4′4′-diaminodiphenylether complex with desoxyribonucleic acid, and 2-nitrofluorene.

ACKNOWLEDGMENT

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American Association for Cancer Research, Inc.

Special Meeting of the Board of Directors

October 13, 1946

The Board of Directors met at 1:45 p. m. in The Sterling Hall of Medicine of Yale University at 333 Cedar Street, New Haven 11, Connecticut. The meeting was called by order of the Chairman and notices dated October 3, 1946 were mailed to all of the Directors. Present were Directors J. C. Aub, E. V. Cowdry, Jacob Furth, W. U. Gardner, M. J. Shear, and Shields Warren.

The minutes of the previous meeting as published in *Cancer Research*, 1946, volume 6, pages 505-508 were approved.

Report of Officers

The receipt of a gift in the amount of five dollars from Mr. and Mrs. Eugene E. Little "in memory of Elizabeth Hastings" was announced. The Board directed the Acting Secretary to record the gift in the minutes and to express to Mr. and Mrs. Little the gratitude of the Association.

Report of Committees

Journal Committee.—Chairman M. J. Shear inquired as to the respective duties of his committee and the Editorial Committee of the journal. It was pointed out that the Editorial Committee is independent of the Association and represents the owner of the journal, whereas the Journal Committee represents the Association as one of the several contributors to the support of the journal. The Board concurred in the opinion that the Committee is to anticipate eventual assumption of the management of the journal. The Directors expressed the view that in the light of its contribution to the support of the journal the Association is entitled to a financial report. The hope was expressed that the Committee might be able to make such a report at the next meeting of the Board.

Unfinished Business

The Chairman of the Board reported that he is continuing the investigation suggested by the Board of the desirability and possibility of the Association's becoming affiliated with the Federation of American Societies for Experimental Biology. He promised to report fully at a later meeting.

Inasmuch as the war ended more than a year ago, and the Association's members now in the military services are looking forward to military careers, it was voted that "the exemption of members in the armed forces from payment of dues be terminated as of January 1, 1947."

The question of publication prior to the annual meeting of abstracts of the papers to be presented was again considered, but without decision. It was voted to defer action and to consider the question at a later meeting.

New Business

The chairman announced the appointment of Dr. Charles Huggins to the chairmanship of the Local Committee in charge of arrangements for the forthcoming annual meeting. It was also announced that the Federation will meet in Chicago, May 18-22, 1947. After some discussion it was decided to delegate to the Local Committee the choice of a date for the meeting, with the specification that it immediately precede or immediately follow the Federation's meeting.

A suitable designation of papers read at annual meetings at the invitation of the Program Committee was discussed. The Directors agreed that the program should indicate such papers and speakers as "by invitation of the Program Committee."

The Chairman suggested the desirability of having prepared an account of the history of the Association. The Board agreed, and authorized the Chairman to invite a qualified member to serve as historian of the Association.

The Chairman reported that all the Directors had in response to his letter expressed themselves in favor of the Association's affiliation with the National Society for Medical Research whose objective is "to advance and encourage research and teaching in Biology and Medicine . . . by informing the public regarding the necessity, the humane character, and the accomplishments of animal experimentation." It was voted that the Association join the Society and contribute to it the sum of fifty dollars.

Dr. E. V. Cowdry, Chairman of the International Cancer Research Congress Committee, reported that with one exception the members of his committee are in favor of holding an international congress. His committee proposes that the congress be held in St. Louis in September 1947. After discussion of financial and other problems related to arranging a congress, a motion was made "That the President be authorized to invite the Union Internationale Contre la Cancer in conjunction with the American Association for Cancer Research, Inc. to hold an International Cancer Research Congress in September 1947 in the city of St. Louis." The motion was seconded and voted, with one director registering preference for 1948. It was then voted that Dr. E. V. Cowdry be nominated for the office of President of the Congress.

The possibility of prize awards to investigators in cancer research was briefly discussed. The Board directed the Chairman to appoint a committee to report to the Board

if and when any funds for prize awards become available and the Association is asked to suggest recipients or supervise awards.

In view of the cancer research bill debated in the last Congress and of proposals to introduce cancer bills in the new Congress, various aspects of the question of federal support of cancer research received extended discussion. It was voted that "The Board of Directors of the American Association for Cancer Research recognizes that under present circumstances the progress of studies on the cause, prevention, and treatment of cancer is impeded by the limitations of available funds and therefore recommends the further participation of the federal government in the support of cancer researches, and further recommends that this Association express an opinion on matters of policy in connection therewith." It was agreed, however, that no further action should be taken by the Board without the approval of the membership of the Association.

It was also voted that the Committee on Cancer Research, its Organization and Support formulate questions concerning federal support of cancer research to be submitted to the members of the Association after approval by the Board.

It was suggested that the Board meet again when the results of the proposed questionnaire have been tabulated.

It was voted "That the minutes of this meeting be published in Cancer Research and that the Association bear the expenses of publication."

The meeting adjourned at 5:15 p. m.

William U. Gardner, *Chairman*

Charles W. Hooker, *Acting Secretary*

Abstracts

Reports of Research

On the Sensitivity of Different Tissues in Street Strain Mice to 9,10-Dimethyl-1,2-Benzanthracene. ENGELBRETH-HOLM, J., and RASK-NIELSEN, R. [Univ. Inst. of Path. Anat., Copenhagen, Denmark] *Cancer Research*, **7**: 129-133. 1947.

Small quantities (0.02 mgm.) of 9,10-dimethyl-1,2-benzanthracene were injected into the thymus, lymph nodes, subcutaneous tissue, mammary gland, testis, lung, spleen, kidney, and bone marrow of partly inbred mice of the Street strain. Lymphosarcomatous tumor of the thymus without other leukemic changes was noted in nearly all cases. Leukemia was observed following injections as follows: into the thymus, in 8 of 68 mice; into a lymph node, in 2 of 54; into the lung, in 2 of 52; and into the kidney, in 1 of 19. Aside from a single pulmonary adenoma, no other growths were encountered. Injection into the other organs mentioned failed to cause local tumor formation. The controls exhibited 2 cases of mammary carcinoma but no leukemia.

It is concluded that the thymus is more susceptible to the direct application of 9,10-dimethyl-1,2-benzanthracene than are other tissues investigated.—E. B. B.

The Induction of Neoplasms in Five Strains of Rats with Acetylaminofluorene. DUNNING, W. F., CURTIS, M. R., and MADSEN, M. E. [Wayne Univ. Coll. of Med., and Detroit Inst. for Cancer Research, Detroit, Mich.] *Cancer Research*, **7**:134-140. 1947.

Fifty rats of 5 different inbred lines were fed an average of 3.4 mgm. daily of 2-acetylaminofluorene in Sherman's mash (66% whole wheat flour, 33% whole milk powder and 1% salt). After an average of 353 days, 41 neoplasms were observed, 15 of which were malignant, 1 not known and the remainder probably benign. Tumors of the liver were most frequent including 30 benign and malignant neoplasms involving primary liver cells, bile duct epithelium or stroma. The other neoplasms included 5 bladder papillomas and squamous cell cancers, 4 mammary adenocarcinomas, 1 squamous carcinoma of the ear and 1 unknown. Strain differences were observed both in average survival period, and in the organ and tissue which responded to this chemical.—Author's abstract.

The Production of Tumors in the Liver of Rats Fed *m'*-Methyl-*p*-Dimethylaminoazobenzene. CORTELL, R. [Yale Univ. Sch. of Med., New Haven, Conn.] **7**:158-161. 1947.

Rats fed *m'*-methyl-*p*-dimethylaminoazobenzene in a semi-synthetic diet low in protein and riboflavin for 39 and 46 days and then followed for 150 days without the dye, never developed any liver tumors whether the diet during the dye-free period was the semi-synthetic one or fox chow.

Rats that were fed the azo dye in the semi-synthetic diet for 69 days, and then placed on the dye-free diet developed a high incidence of tumors. Approximately 93% of the animals placed on fox chow following the dye feeding period had tumors, while 80% of the animals maintained on the semi-synthetic diet developed tumors. The tumors of the animals receiving fox chow appeared earlier and were more malignant as evidenced by the greater incidence of lung metastases.

The histopathology of liver tumors produced by *m'*-methyl-*p*-dimethylaminoazobenzene was found to be similar to that of tumors produced by the parent dye, *p*-dimethylaminoazobenzene, namely liver cell carcinoma, bile duct cystadenoma and cholangioma.—Author's abstract.

***p*-Dimethylaminoazobenzene Carcinogenesis with Purified Diets Varying in Content of Cysteine, Cystine, Liver Extract, Protein, Riboflavin, and Others Factors.** HARRIS, P. N., KRAHL, M. E., and CLOWES, G. H. A. [Lilly Research Labs., Eli Lilly & Co., Indianapolis 6, Ind.] *Cancer Research*, **7**: 162-175. 1947.

In most of these experiments the carcinogen was administered at a level of 0.9 mgm. per gm. of diet, although in a few the level was reduced to 0.6 mgm. per gm. Administration of the compound was terminated only with the death of the animals. The rats were palpated at weekly intervals and graphs showing the cumulative tumor percentage plotted against time are shown for most of the diets presented. Retardation of carcinogenesis was effected by addition to the diet of the following substances: 1% of cystine; 1% of cysteine; 5 to 8 mgm. of riboflavin per kgm. of a 20% casein diet; and 3% of liver extract added to a diet favorable to early carcinogenesis. Addition of 3% of liver extract to a protective diet did not result in further protection. Fifteen per cent of liver extract offered much less protection than did 3%. The following substances had no appreciable effect upon carcinogenesis under the conditions of the experiment: choline; inositol; lipocaic; *p*-aminobenzoic acid; pantothenic acid; and succinic acid.—Authors' abstract.

Survey of Hypnotics in General with Regard to Pulmonary Tumor Induction in Mice. LARSEN, C. D. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **7**:5-8. 1946.

Urethane was found to be as effective in the production of pulmonary tumors in strain A mice when small amounts were added to the drinking water as when necrotizing doses were given by intraperitoneal injection. A series of barbituric acid derivatives employed at or near the maximum tolerated dosage level was tested and found not to be carcinogenic to the pulmonary tissue of mice. Likewise a series of compounds including urea, thiourea,

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ethanol, ethylal, methylal, sulfonal, trional, chloral hydrate and paraldehyde proved to be ineffective in increasing the incidence or number of pulmonary tumors in strain A mice.—R. A. H.

The Milk Factor in Blood. Graff, S., Randall, H. T., Carpenter, G. E., and Haagensen, C. D. [Coll. of Physicians and Surgeons, Coumbia Univ., New York, N. Y.] *Science*, **104**:289. 1946.

Blood from females of the high mammary cancer Paris strain of mice appears not to be a rich source of the milk factor. Young females of the C57 strain were used to test for the presence of the factor. These animals normally develop no spontaneous mammary carcinomas, but are susceptible to the milk factor as judged by the fact that following foster nursing on high mammary cancer strain females, 76.1% develop mammary cancer.

In the tests 198 young C57 females were each given a single subcutaneons injection, usually 0.2 cc., of various fractions of blood from the Paris strain females. These fractions included whole serum, fat-free serum, serum globulins and albumins, neutral fats, washed red cells, and hemolyzed and dehydrated red cells. No mammary tumors developed in the test mice, which were observed until they died at a mean age of 24.4 months. Among 20 additional mice injected with whole blood, 1 developed a mammary tumor.—R. B.

Observations on Radiation-Induced Lymphoid Tumors of Mice. Kaplan, H. S. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research*, **7**:141-147. 1947.

Fractionated whole-body x-ray treatment was given to Strain A mice in groups of various ages ranging from 2 weeks to 6 months. The experiment was terminated about 1 year after irradiation, at which time the incidence of lymphoid tumors was 26.4% among 38 surviving animals treated at 1 month of age or earlier, and only 6.0% among 117 mice 2 months of age or over at the time of irradiation. The average induction time in the combined younger group was 207.9 days, against an average of 225.4 days in the older age groups. The slightly higher incidence noted in male mice was not considered significant.

Strain A mice 2 to 3 months of age were thymectomized or splenectomized and given fractionated whole-body radiation. There were only 2 lymphoid tumors among 45 thymectomized-irradiated mice (1.7%). The induction times for these neoplasms were 379, 437, and 231 days, respectively. Intact irradiated controls of the same age had a low incidence of lymphoid tumors (7.4%) with an average induction time of 229 days. The age at which radiation is administered must be properly selected in order to obtain an optimum tumor incidence in control groups for purposes of comparison.

The morphology of these tumors is similar to that previously described in estrogen- and carcinogen-induced lymphoid tumors. The thymus is the most commonly involved organ, and the disease spreads to the lungs, liver, spleen, and kidneys more often than to the peripheral lymph nodes. Histological examination of mice killed at the end of the latent period has revealed instances in which one lobe of the thymus contained a definitive lymphoma while all other tissues of the body, including the opposite thymic lobe, were microscopically free of disease. These observations suggest that radiation-induced lymphoid tumors arise locally rather than systematically, and that the thymus is their preferential site of origin.

Incidental observations on induced ovarian tumors and on the reduced incidence of spontaneous mammary tumors among these irradiated strain A mice are also presented.—Author's abstract.

Neutral B-Glycerophosphatase Activity in Normal and Neoplastic Tissues. Greenstein, J. P. and Leuthardt, F. M. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **7**:1-3. 1946.

Extracts of several normal and neoplastic mouse tissues were tested for phosphatase activity at pH 6.7 to 6.9 using β-glycerophosphate as the substrate. Of the tissues tested normal intestinal mucosa exhibited the greatest activity and normal thymus the least. With the exception of a thymoma, the activity of the mouse tumors was lower than that of the corresponding normal tissue although 2 tumors for which there were no control normal tissues tested showed rather higher activities than most of the normal tissues. Dialysis did not decrease the phosphatase activity of the tissue extracts, nevertheless the addition of Mg^{++}, and to a lesser extent Na^{+}, to dialyzed extracts increased their activity.—R. A. H.

Enzymatic Desamination and Dephosphorylation of Ribosenucleic and Desoxyribosenucleic Acids. Greenstein, J. P., Carter, C. E., Chalkley, H. W., and Leuthardt, F. M. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **7**:9-27. 1946.

The ability of extracts of several normal and neoplastic rat and mouse tissues to liberate ammonia nitrogen and inorganic phosphate from ribose- and desoxyribosenucleates and from purines, pyrimidines, nucleosides and nucleotides was studied Adenylic guanylic, cytidylic and uridylic acids were dephosphorylated, and adenylic and guanylic acids desaminated by most tissue extracts, whereas cytidylic acid and cytidine were desaminated to an appreciable extent only by extracts of mouse kidney, and uridylic acid was not desaminated by any of the tissue extracts studied. Time course curves using the several substrates are given for normal rat liver, kidney, spleen, brain, pancreas and muscle and for mouse liver and kidney. These show, in general, greater desamination and dephosphorylation by extracts of kidney and spleen, less by those of liver and relatively little by those of brain, pancreas and muscle. There was essentially no difference in the rate at which extracts of primary hepatoma and of normal liver desaminated and dephosphorylated the 2 nucleates although extracts of a transplanted hepatoma were considerably more active and those of fetal liver generally less active in both respects. In extracts of other tumors studied, desoxyribonsenucleates were attacked to only a very small extent although ribosenucleate was readily attacked. The effects on the enzymic degradation of the 2 nucleates of dialysis and of the addition of various salts to the dialyzed extracts of normal tissues is studied rather extensively, and there is a considerable discussion on the general picture of nucleic acid metabolism in tissue extracts.—R. A. H.

Studies on the Enzymatic Degradation of Nucleic Acids. Carter, C. E., and Greenstein, J. P. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **7**:29-45. 1946.

A method is described for following the enzymatic degradation of nucleic acids by the determination of cer-

tain of the dialyzable components by means of chemical and spectrophotometric technics. The effects of various electrolytes upon the rate of degradation of the 2 general types of nucleic acid was studied. It was found that, although many different ions were capable of activating purified desoxyribonuclease to varying degrees, Mn++, Ba++and Mg++, were the most effective in this respect. While no electrolyte is essential for the active degradation of ribosenucleic acid by ribonuclease, both Mg++ and Na+ were found to increase this degradation greatly, and it was found that both of these ions with no enzyme present caused a breakdown of non-dialyzable ribosenucleic acid to dialyzable fragments. This fragmentation occurred without the liberation of titrable acid groups while the degradation of both types of nucleic acid by their respective enzymes was found to be associated with the liberation of titrable acid groups. Spectrophotometric studies revealed evidence suggesting that some, but not all, extracts of rat hepatoma #31 exhibited an increased oxidation of the purine and pyrimidine components of both types of nucleic acid. Studies determining the development of color with the Folin phenol reagent following the incubation of extracts of normal rat liver and hepatoma #31 with the 2 nucleates indicate that "the metabolism or desoxyribosenucleic acid in hepatoma #31 differs strikingly from that in normal liver in failing to develop chromogenic material, presumably xanthine or guanine, whereas for ribosenucleic acid digests with the tumor produce more chromogenic material."—R. A. H.

The Metabolism of *N,N*-Dimethyl-*p*-Aminoazobenzene and Related Compounds. Kensler, C. J., Magill, J. W., and Sugiura, K. [Memorial Hosp., New York, N. Y.] *Cancer Research*, **7**:95-97. 1947.

The observations of the Wisconsin group that *N, N*-dimethyl-*p*-aminoazobenzene (DMB) is converted in the rat, at least to some extent, to *p*-aminoazobenzene (AB) have been confirmed. However, only a small percentage of the DMB fed is found as AB. Feeding *N, N*-diethanol-*p*-aminoazobenzene (DE-ol-B), in contrast to our findings with *N, N*-diethyl-*p*-aminoazobenzene (DEB), led to the accumulation of only small amounts of AB. Liver slices have been found to destroy DMB and AB *in vitro*. No conversion of DMB to AB was detected. Liver tumor slices destroyed DMB to a much lesser extent. Ground brown rice has been found to demethylate some DMB (in cottonseed oil) when mixed with the diet. Heating the brown rice at 90° C. for 5 days before mixing, inhibited this action.—Authors' abstract.

The Proteolytic Activity of Normal and Pathological Human Thyroid Tissue. De Robertis, E., and Nowinski, W. W. [Inst. of General Anat. and Embryol., Faculty of Med., Univ. of Buenos Aires, Buenos Aires, Argentina] *J. Clin. Endocrinol.*, **6**:235-246. 1946.

Under standardized conditions the proteolytic activity of "normal" thyroid tissue amounted to 0.37 — 0.57 mgm. of tyrosine and tryptophane (expressed as mgm. equivalents of tyrosine). Measurements were made photometrically by estimating the tyrosine and tryptophane liberated from the substrate edestin with the use of the phenol reagent of Folin-Ciocalteu. The "normal" thyroid tissue was obtained from 10 patients without clinical symptoms of the disease or altered BMR. In 4 of these cases the samples of normal histological appearance were selected at a distance from simple encapsulated adenomas which were present in the gland.

In 11 cases of severe toxic goiter the proteolytic activity was 96% above that of the normal gland. In 2 cases of toxic nodular adenoma the proteolytic activity of the adenomatous tissue was about 100% greater than that of adjacent tissue from the same gland. In mild toxic goiters kept under control by treatment with iodine, the proteolytic activity was 28% below normal values. The writers conclude that the proteolytic enzyme system plays a significant role in the release of colloids and in the pathological function of toxic and simple goiters. They believe that the therapeutic effect of iodine is due partly to inhibition of the proteolytic system responsible for the breakdown of the thyroglobulin molecule, and in this manner prevents the release of hormones from the follicles. The inhibition is supposed to involve direct iodinization of the proteolytic enzyme in the gland.—J. B. H.

Adenocarcinoma of the Thyroid with Hyperthyroidism and Functional Metastases. I. Studies with Thiouracil and Radio-Iodine. Leiter, L., Seidlin, S. M., Marinelle, L. D., and Baumann, E. J. [Montefiore Hosp., and Memorial Hosp., New York, N. Y.] *J. Clin. Endocrinol.*, **6**:247-261. 1946.

In 2 cases of hyperthyroidism with adenocarcinoma of the thyroid gland the presence of functional metastases was demonstrated by the uptake of radioactive iodine by metastatic areas. In one of the patients the thyroid tissue in the neck was non-functional, indicating that metastases were responsible for the hyperthyroidism. Thiouracil suppressed the production of hormones in the metastases and during its period of maximal effect doubled the urinary excretion of radioactive iodine.—J. B. H.

Urinary 17-Ketosteroids in Metabolism. II. Partition of Gonadal and Adrenocortical Normal Derivatives of Normal, Endocrine and Cancerous Patients. Salter, W., Cahen, R. L., and Sappington, T. S. [Yale Univ. Sch. of Med., New Haven, Conn.] *J. Clin. Endocrinol.*, **6**:52-76. 1946.

In 30 normal subjects and 48 with various abnormalities, a study was made colorimetrically of the total urinary 17-ketosteroids per 24 hours and of 2 fractions, the 3-cis (β) and 3-trans (α) hydroxyl. A small amount of the data pertains to patients with cancer. Thus, the values for the total 17-ketosteroid excretion were low in cancerous patients who had no tumors of the adrenal glands or gonads and who were not cachetic or malnourished. The titers of steroids were normal in a precociously pubertal 3½ year-old girl with a granulosa-cell tumor of the ovary. In virilism with adrenal hyperplasia the total values were as much as several times the normal range. With a tumor of the adrenal cortex the relative value of the cis (β) fraction tended to be disproportionately high.—J. B. H.

A Heterologous Principle in Human Cancerous Liver: Its Effect upon the Brown-Pearce Tumor. Penn, H. S., and Jacobsen, E. M. [Univ. of California, Los Angeles, Calif.] *J. Immunol.*, **54**:17-33. 1946

This article is a preliminary report of results of investigation of a possible growth-inhibiting effect exerted upon the Brown-Pearce carcinoma of the rabbit by an acetone-soluble lipoid obtained from human sources, "chiefly cancerous liver." The nature of the human cancerous material is not stated and a number of technical details, as well as some results of a control series are left for later

publication. Although the assumption of an immunological reaction remains to be proved the term "antigen" is used for the lipoid extract of human cancer or of human cancerous liver, and the term "antiserum" is used for the plasma fraction obtained from rabbits injected with the antigen. The terms "normal antigen" and "normal serum" refer respectively to lipoid extracted from non-cancerous livers and to identically processed plasma from uninjected rabbits. The cancer antigen was made by evaporation of the acetone-soluble fraction of an extract of cancerous human liver and then trituration of 1 gm. of the lipoid residue with 10 cc. horse serum. The horse serum was added as a "schlepper", which is defined as "a term applied to a protein when combined with a non-antigenic substance (hapten)." Immunization schedules are given for the preparation of the antiserum. New Zealand white rabbits and pigmented Himalayan or other breeds were used. The animals were inoculated intraocularly with the Brown-Pearce carcinoma of varying degrees of malignancy. Ten to 18 days after inoculation with the tumor the rabbits were treated by injections of the anti-cancer anti-serum and antigen, or "normal antigen" and normal rabbit serum. (Experiments are now in progress in which a series of animals is being injected with normal antigen and normal antiserum).

Observations were made on the rapidity of growth of the transplanted tumor, incidence of regressions, extent of metastases, and histological appearances of the growths or nodules. The usual variability of the malignancy of the Brown-Pearce carcinoma was encountered. With a sluggish tumor of low malignancy, in which there was a high incidence of regression, no difference could be detected between the rabbits treated with antiserum and antigen, and the control or "normal groups." With Brown-Pearce carcinoma of high malignancy a definite suppression of growth was observed in the primary tumor and a low incidence of dissemination in the rabbits treated with antiserum and antigen. The figures are not treated statistically. The authors conclude tentatively that the acetone-soluble antigen obtained from human cancerous tissue or its antiserum or both may contain substances that inhibit the growth of Brown-Pearce carcinoma.—S. B.-J.

Changes in the Succinoxidase Activity of Livers from Rats during the Development of Hepatic Tumors on Feeding *p*-Dimethylaminoazobenzene. Hoch-Ligeti, C. [Royal Cancer Hosp., London, England] *Cancer Research*, **7**:148-157. 1947.

The succinoxidase activity of rat liver slices and homogenates was studied during the course of development of hepatic tumors due to feeding of *p*-dimethylaminoazobenzene. The succinoxidase activity of the homogenates of livers from rats not receiving *p*-dimethylaminoazobenzene varied slightly with the diet; it was lowest with a high-fat diet and highest with a rice diet containing 6% casein, where the percentage of fat was lowest. The 0_2 uptake of homogenates of liver, with and without excess of cytochrome C, from rats receiving *p*-dimethylaminoazobenzene was only sightly lower than the control irrespective of whether the animal had developed a liver tumor or not. The tumors themselves showed generally a low succinoxidase activity. Slices of liver from animals on *p*-dimethylaminoazobenzene had lower succinoxidase activity than the controls. The succinoxidase activity was further depressed on the development of tumors and was very low in tumor tissue. In one series of rats where the addition of milk largely prevented the development of liver tumors the succinoxidase activity of liver slices or homogenates did not differ from rats on the control diet. The addition of azobenzene to a rice-carrot diet had no effect on the succinoxidase activity of the homogenates or slices of livers. The discrepancy between the values for succinoxidase when using homogenates, or slices, of the same liver from animals fed on *p*-dimethylaminoazobenzene might be explained by a different accessibility of the parts of the enzymic system or by assuming an intracellular inhibition in these livers.—Author's abstract.

Further Data on the Action of Azo-Compounds in Rats and Mice. Kirby, A. H. M., *Biochem. J.*, **40**: lvi. 1946.

Diazoaminobenzene (DAAB) did not produce neoplasia when injected or fed to mice but cancer developed in 2 mice when it was painted on the skin. *p*-Aminoazobenzene caused liver tumors in rats when added to a diet containing potato, but not when the potato is replaced by starch. Starch showed no protection against liver tumors when *N*,*N*-dimethyl-*p*-aminoazobenzene (DAB) was added to the diet. *N*,*N*-diethyl-*p*-aminoazobenzene (EAB) did not induce liver tumors.—I. H.

A Nutritional Concept of Cancer. Lederberg, J. [Yale Univ., New Haven, Conn.; and Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Science*, **104**:428. 1946.

The author proposes a mechanism to account for the origin of malignant cells which is based on an analogy with the behavior of the "leucineless" mutant of the mold, Neurospora. Field strains of Neurospora grow on a medium containing only sugar, salts, and biotin. The leucineless mutant, produced by ultraviolet treatment, requires the addition leucine, and the amount of growth is controlled by the amount of leucine added to the basal medium. Occasionally parts of a Neurospora mycelia growing on a medium containing a limited amount of leucine will begin growing faster than the rest and will overgrow the culture until the sugar is exhausted. Genetic analysis shows that this "adaptation" depends on the mutation or reversion of the leucineless gene to an allele capable of mediating the synthesis of leucine.

The analogy with cancer is as follows: The rather complex nutritional requirements of normal cells of higher organisms are supposed to have arisen by a series of mutations similar to those produced experimentaly in Neurospora, and control of growth is assumed to depend on amounts of essential substances made available to the cells. A mutation allowing a cell to synthesize an essential metabolite otherwise available only in limited and regulatory amounts would endow such a cell with the capacity for uncontrolled growth. Although the Neurospora work suggests mutation as the mechanism bringing about such a change, virus infection, by providing a missing link for a blocked enzyme system, could play a corresponding role. —R. B.

The Effect of Biotin upon *p*-Dimethylaminoazobenzene Carcinogenesis. Harris, P. N., Krahl, M. E., and Clowes, G. H. A. [Lilly Research Labs., Eli Lilly & Co., Indianapolis, Ind.] *Cancer Research*, **7**:176-177. 1947.

A 15% liver extract diet had given less protection against *p*-dimethylaminoazobenzene carcinogenesis than had one of 3%. This effect was attributed to biotin, and to test this interpretation another experiment was run with 4 diets: (1) control (favorable to early carcinogenesis); (2) control plus 0.3 γ biotin per gm. of diet; (3) control plus 3% of liver extract; and (4) control plus liver extract and biotin at the levels in (2) and (3). The biotin level in diets and (2) and (4) was approximately that in the 15% liver extract diet. The curves of tumor development for diets (1) and (3) were similar to those of the earlier control and 3% liver extract diets, and that for diet (4) paralleled that obtained with the 15% liver extract diet. Addition of biotin to the control diet did not accelerate tumor development.—Author's abstract.

The Effect of Diet Containing Dried Egg Albumin upon *p*-Dimethylaminoazobenzene Carcinogenesis. Harris, P. N. [Lilly Research Labs., Eli Lilly & Co., Indianapolis, Ind.] *Cancer Research*. 7:178-179. 1947.

Development of liver tumors in rats fed a diet containing 15% of dried egg albumin and 0.06% of *p*-dimethylaminoazobenzene was greatly retarded as compared with the development of liver tumors in rats on a diet favorable to tumor growth. The degree of protection was greater than that provided by casein and riboflavin, and appeared to be slightly increased by the addition of 3% of liver extract.—Author's abstract.

Creatine Metabolism in Relation to Pituitary Tumours. Cumings, J. N. [Biochem. Lab., Nat. Hosp., London, England] *Brain*, **67**:265-269. 1944.

Urine from 8 patients with a chromophobe adenoma and 16 with an acidophil adenoma of the pituitary have been examined for creatine and creatinine while on a meat- and fish-free diet. All patients with a chromophobe adenoma have shown a normal or slightly raised excretion of creatinine and on some days small amonts of creatine. All the 16 cases of acromegaly showed a definite but variable amount of creatine with an increased amount of creatinine. A creatine tolerance test (1 gm. creatine by mouth) is described. In acromegaly the fasting level of blood creatine is higher than normal, rises rapidly and tends to fall in 3 hours, whereas in a patient with a chromophobe adenoma the fasting figure is within normal limits, rises rapidly and tends to remain raised even after 3 hours. The urinary creatine is much higher in the acromegalic patient.—E. L. K.

The Chemistry of Cerebral Tumours and of Cerebral Cyst Fluids. Cumings, J. N. [Biochem. Lab., Nat. Hosp., London, and Neuro-Surg. Unit, E. M. S., England] *Brain*, **66**:316-321. 1943.

Analyses of fluids were made for water content, potassium, sodium, chlorides, calcium, and phosphorus (acid-soluble, nucleoprotein, phospholipoid). Cysts containing little or no nucleoprotein and phospholipoid are perhaps derived from hemorrhages. The amount of nucleoprotein and phospholipoid in the majority of cerebral cysts suggests that they are formed by degeneration of tumor tissue and perhaps of brain tissue also.—E. L. K.

The Effect of Urethane on Lymphatic Leukemia in Rats. Murphy, J. B., and Sturm, E. [Rockefeller Inst. for Med. Research, New York, N. Y.] *Science*, **104**:427. 1946.

Urethane (ethyl carbamate) inhibits strongly the development of transplanted lymphosarcoma or leukemia in rats. The tumor used in this study develops as generalized lymphatic leukemia if injected intraperitoneally, causing death in 8 to 12 days; or as localized lymphosarcoma when injected into the subcutaneous tissue of the groin, causing death in 16 to 21 days.

Among 50 intraperitoneally inoculated rats, given 50 to 100 mgm. of urethane per 100 gm. of body weight, repeated 4 times a week, only 3 developed leukemia (6%). Among 41 controls 33 developed fatal leukemia (80.4%). Among 30 rats inoculated in the groin and given the urethane treatment only 9 (30%) developed progressive tumors, while 26 of the 30 controls (86.6%) died of lymphosarcoma.

The adrenals of the urethane-treated rats were found to weigh about 33% more than those of normal untreated rats. This is thought to be significant in view of the fact that it has been demonstrated previously that adrenalectomy renders rats more susceptible to transplanted leukemia (*Science*, **98**:568. 1943), while adrenal cortical and pituitary adrenotropic hormones render them less so (*Science*, **99**:303. 1944).—R. B.

Trophoblast Elements in Cancer. Krebs, E. T. Jr., and Gurchot, C. [Univ. of California Med. Sch., San Francisco, Calif.] *Science*, **104**:302. 1946.

According to information reviewed by the authors certain tumors known to contain trophoblastic elements, such as chorionepitheliomas, many ovarian and testicular cancers, and malignant teratomas, produce prolan in sufficient quantities to give a positive Aschheim-Zondek reaction. Previous attempts to demonstrate prolan derived from most other types of tumors have failed. In the present communication, however, it is reported that prolan has been detected in urine from a human male with nongenital cancer by a technic involving chromatographic adsorption and testing on *Xenopus laevis*, the South African clawed toad. Controls of the same age were negative. This result is thought to support the idea that all cancers contain trophoblastic elements as the constant malignant component.—R. B.

Differential Blood Counts in Certain Adrenal Cortical Disorders (Cushing's Syndrome, Addison's Disease and Panhypopituitarism). De la Balze, F. A., Reifenstein, E. C., and Albright, F. [Med. Service of Massachusetts General Hosp., and Harvard Med. Sch., Boston, Mass.] *J. Clin. Endocrinol.*, **6**:312-319. 1946.

In view of reports in the literature that treatment of animals with adrenotrophins or with certain adrenocortical compounds increased the absolute number of neutrophils and decreased the absolute number of lymphocytes in the blood stream, De la Balze and his co-workers studied the quantities of neutrophils, lymphocytes, and total number of leukocytes in 3 clinical conditions which involve abnormalities of the adrenal cortex.

In 10 women with Cushing's syndrome there was an increase in the total leukocyte count and in both the absolute and the relative neutrophil count, plus a decrease in the relative (and usually in the absolute) lymphocyte count. These hematological changes are similar to initial events in the Adaptation Syndrome as described by Selye and are the opposite of the alterations (a relative neutropenia and a relative lymphocytosis) observed in 12 women

and 8 men with Addison's disease and in 8 women and 12 men with panhypopituitarism. Suggestions are made that Cushing's syndrome is a state of hyperfunction of the adrenal cortex with regard to the production of those hormones which affect carbohydrate metabolism; that a persistent lymphopenia should be sought as corroborative evidence in the diagnosis of Cushing's syndrome; that the increased sensitivity to infection in Cushing's syndrome is due to depletion of lymphoid tissue from which immune globulin is normally released; and that the increased sensitivity to infection in Addison's disease and in panhypopituitarism is due to a lack of the adrenal cortical secretions which release immune globulin from lymphoid tissue.—J. B. H.

Studies on Antireticular Cytotoxic Serum. I. Introduction and Review of the Literature. STRAUS, R. [Cedars of Lebanon Hosp., Los Angeles, Calif.] *J. Immunol.*, **54**:151-154. 1946.

Articles on the production and experimental and clinical use of antireticular cytotoxic serum (ACS) have been numerous in the Soviet medical literature since 1924. The investigations of Bogomolets and his associates since 1939 and the unusual and remarkable claims made for the beneficial effects of treatment with ACS in many different diseases including cancer, have brought this serum to special attention in the past 3 years. The author prepared this review of 20 articles as an introductory note for reports of his series of experimental investigations of the serum.—S. B. J.

Studies on Antireticular Cytotoxic Serum. II. Preparation and Titration of the Serum and Study of Its Serological Properties. STRAUS, R., RUNJAVAG, M., ZAITLIN, R., DUBOFF, G., and SWERDLOW, H. [Cedars of Lebanon Hosp., Los Angeles, Calif.] *J. Immunol.*, **54**: 155-162. 1946.

Marchuk's technic with slight modifications was used to prepare antireticular cytotoxic serum. The antigen used for immunization of rabbits and goats was made from spleen and bone marrow obtained from human cadavers not more than 12 hours postmortem. These tissues removed aseptically were weighed, minced, mixed in proportion of 4 parts spleen to 1 part of bone marrow, ground in a mortar with 5 volumes of saline and allowed to stand 30 minutes at room temperature. The minced tissue and saline were then centrifuged at 1,000 r.p.m. for 5 minutes and the cloudy supernatant was used as antigen. Animals were injected within 1 to several hours after the tissues were secured. The use of fresh antigen was essential. The injections were made intravenously at intervals of 3 days in a series of 6 treatments with amounts of tissue increasing from 0.25 gm. to 6 gm. At first animals under immunization died of anaphylactic shock. This was largely avoided by the schedule of injections worked out by the authors. To obtain serum, immunized animals were bled usually on the fourth to seventh day after the last injection.

The titer of the ACS was determined by a complement fixation test in which the immune serum, as antibody, was set up in serial dilutions, against the antigen (saline extract of human bone marrow and spleen). Details of all the titration methods are given. The usual titers of antihuman ACS prepared in rabbits were 1:640 to 1:1,280; in goats, 1:160 to 1:320.

The antihuman ACS prepared in rabbits and goats was essentially species specific, although there were slight reactions with antigens from mouse, rat and rabbit. The antibody responsible for these cross-reactions was removed by absorption with sheep RBC, and was thought a Forssman type of antibody. Hemolysins were usually not present in the ACS in titers greater than 1:20. Hemagglutinins were present in some specimens of ACS in titers up to 1:640. These antibodies were removed by absorption with human RBC without materially reducing the titer of the ACS.

Liquid ACS was kept at 4°C. for several months, with slight loss of titer, and considerable loss of titer in a year. Lyophilized ACS was more stable than the liquid serum.—S. B. J.

Studies on Antireticular Cytotoxic Serum. III. Effect of ACS on the Healing of Experimentally Produced Fractures in Rabbits. STRAUS, R., HORWITZ, M., LEVINTHAL, D. H., COHEN, A. L., and RUNJAVAC, M. [Cedars of Lebanon Hosp., Los Angeles, Calif.] *J. Immunol.*, **54**:163-177. 1946.

The experiments reported in this paper were undertaken to test the statement of Bogomolets and others that ACS in small doses stimulates, and in larger doses depresses, the healing of fractures. The antirabbit ACS used was prepared in goats and standardized according to the method described in the preceding paper by Straus and associates. Under nembutal anesthesia, the right radius and ulna of healthy rabbits were fractured with a modified Grattan osteoclast. The fractures were reduced, the limbs encased in Castex, and x-ray and other studies were made of healing, callus formation and strength of union. Bones removed from animals killed at various periods after the fractures had been set and after treatment with ACS were broken in an apparatus that gave values for "breaking strength." At the start there were 156 animals in the test and control groups in 3 successive experiments. All were kept under similar conditions. Seventy-four animals were eliminated, giving an effective total of 82. On the third day after the fracture the rabbits in separate groups were injected intravenously with ACS or normal goat serum.

The results were treated statistically. The claim of the Soviet investigators was confirmed. Stimulation of the healing of experimentally produced fractures in rabbits was induced with small, "stimulating" doses of ACS; depression of healing by large, "depressing" doses of ACS. The paper is illustrated with photographs of fractures in various stages of repair and photomicrographs of sections. —S. B. J.

Epidermal Nuclear Size Changes in Methylcholanthrene Induced Carcinogenesis. KRAMER, D. Z. [Washington Univ. Sch. of Med., and Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *Anat. Rec.*, **94**:289-310. 1946.

Measurements were made on nuclei isolated from the epidermis of Swiss mice following painting of 0.6% methylcholanthrene in benzene. In mice given 3 paintings during one week and sacrificed on the 11th day after the first painting, nuclear volume was increased about 50% over that of normal untreated skin. This increase was maintained in mice given 6 paintings over a 2 week period (sacrificed on the 21st day), and in those painted 12 times over 3 weeks and sacrificed on the 31st day. Ben-

zene controls painted 3 times in 1 week showed a 22% increase in nuclear volume which, however, was not maintained, for among mice painted with benzene 12 times over a 3 week period the nuclear volume was slightly smaller than normal.—R. B.

Sex Hormone Effects on Chromosome Size in Leukemic and Normal Lymphocytes of C58 Mice. Biesele, J. J., and Gasic, G. [Carnegie Inst. of Washington, Cold Spring Harbor, L. I., N. Y.] *Cancer Research*, **7**:65-69. 1947.

Testosterone propionate implanted in pellets maintained chromosomes of transplanted lines I or Ia leukemic cells in inguinal nodes and spleens of spayed and intact female C58 mice at the large size found in male hosts with or without pellets. In the absence of exogenous androgen, leukemic-cell chromosomes in intact female hosts were significantly smaller in 2 of 3 experiments and in ovariectomized female hosts were reduced still further in 4 of 4 experiments, toward the size of normal lymphocyte chromosomes in adults.

In contrast, the chromosomes of normal lymphocytes were no larger and perhaps smaller in nonleukemic spayed females bearing hormone pellets than in those bearing none. Ovariectomy alone apparently did not alter the size of normal lymphocyte chromosomes in adult females.—Authors' summary.

Chromosomes in Lymphatic Leukemia of C58 Mice. Biesele, J. J. [Carnegie Inst. of Washington, Cold Spring Harbor, L. I., N. Y.] *Cancer Research*, **7**:70-77. 1947.

The progressive increase in chromosome size from normal lymphoid tissue in adult C58 mice through cases of spontaneous leukemia to lines of long-transferred and highly virulent leukemic cells reverses and overcompensates for the normal ontogenetic reduction of about one-half in lymphocyte chromosome size from the newborn animal to the adult. In this enlargement, however, no doubling in number of microscopically visible strands is seen in chromosomes partly uncoiled with dilute alkali.

Partial digestion of sections by pepsin shrinks the chromosomes of leukemia relatively more than normal lymphocyte chromosomes and thus eliminates most of the size difference between them. If shrinkage occurs because of the removal of pepsin-digestible protein and the contraction of the residual nucleohistone, the difference in chromosome size between normal and leukemic lymphocytes may be attributed largely to the difference in amounts of pepsin-digestible protein.—Author's abstract.

In Vitro Staining and Retardation of Growth of Sarcomata in Mice. Lewis, M. R., Sloviter, H. A., and Goland, P. P. [Carnegie Institution of Washington, Baltimore. Md.; Wistar Inst. of Anat. and Biol., and Sch. of Med., Univ. of Pennsylvania, Philadelphia, Penn.] *Anat. Rec.*, **95**:89-96. 1946.

A total of 114 dyes were administered to tumor-bearing mice of the Bagg albino strain and the authors' inbred black agouti strain. The tumors were sarcomas native to the strains used. Nile blue and its oxazone, fed in 0.1% or 0.4% concentration in the food, stained the living parts of the tumors, and brought about a striking reduction in their growth rate. None of the other dyes tested had either of these effects.—R. B.

Comparative Cytology of Wright's Stained Smears and Histologic Sections in Multiple Myeloma. Erf, L. A., and Herbut, P. A. [Jefferson Med. Coll., Philadelphia, Pa.] *Am. J. Clin. Path.*, **16**:1-12. 1946.

The value of Wright's stained smears of bone marrow is compared with sections of marrow taken at biopsy or autopsy in 7 instances of multiple myeloma. It is concluded that the Wright's stained smears are of greater value than sections. The latter may lead to erroneous diagnoses.—S. H. D.

Inverse Relation of Nuclear and Cytoplasmic Function. Dublin, W. B. [Clyde K. Emery Clin. for Neoplastic and Allied Dis., Los Angeles, Calif.] *Northwest Med.*, **44**:287-288. 1945.

A review of experiments by the author and others, indicating that malignant neoplasms do not exhibit the regular rhythm of nuclear division seen in normal non-neoplastic tissue. The peak of mitosis in non-neoplastic tissues occurs when physiologic function is minimal.—M. H. P.

Surface Measurements of Radioactive Phosphorus in Breast Tumors as a Possible Diagnostic Method. Low-Beer, B. V. A. [Univ. of California Hosp., San Francisco, Calif.] *Science*, **104**:399. 1946.

Following injection into patients of radioactive phosphorus, the radioactivity at the surface of the skin over various types of breast tumors and over axillary and supraclavicular nodes was measured with a Geiger-Müller counter. Over tumors later found to be malignant the radioactivity was found to be 25% or more above that of comparative normal areas. Over benign tumors the difference in activity, between involved and uninvolved tissue, was consistently less than 25%. In a series of 25 patients with breast tumors, preoperative diagnoses based on surface radioactivity measurements were found (by subsequent microscopical examination) to be correct in all but one of the cases.—R. B.

Nouvelles recherches historadiographiques appliquées à l'étude des tumeurs. [New Historadiographic Research Applied to the Study of Tumors.] Lamarque, J. P., and Guibert, H. L. [Anticancer Center, Montpellier, France] *Bull. Assoc. franç. p. l'étude du cancer*, **31**:13-28. 1943.

Complex tumors, *i.e.*, tumors possessing different types of tissue, such as a breast epithelioma with epidermoid metaplasia, a mammary tumor of the dog, and a sudoriparous epithelioma of the scalp with epidermoid ataplasia, have been studied by routine histological technics and historadiography. Certain facts have been established. The chromatin in a state of rest or in kinetic activity is more transparent than the cytoplasm. But if degeneration takes place (picnosis or chromatolysis), the chromatin becomes more opaque to the x-rays. The same is true for atypical or degenerative mitosis. The nucleolus has the same opacity as the cytoplasm when it is a true nucleolus. A false nucleolus is transparent. In cancer the nucleolus remains opaque. The cytoplasm is opaque in cancer as well as normal tissue. But it becomes transparent in lipoidal degeneration. When epithelial cells are in a state of epidermoid metaplasia, the opacity to the x-rays increases. In a mesenchymal tissue the col-

lagen is very opaque; the mucoid substance is transparent; the chondroide tissue more opaque, and the osteoid tissue very opaque. Details are given of the historadiographic aspects of several tissues. In conclusion it is pointed out that interesting facts may be established concerning the physico-chemical structure of tissue elements, but the technic has to be improved, especially in so far as it concerns the quality of the emulsion of the photographic films.—R. J.

Papillary Cystadenomata of the Gizzard of Fowls. Wickware, A. B. [Science Service, Dominion Dept. of Agric., Poultry Path. Lab., Ottawa, Canada] *Canad. J. Comp. Med.*, **9**:34-37. 1945.

Papillary adenoma of the mucous membrane of the gizzard, without marked change in the blood or other organs appeared in 3 of 10 chicks that had been injected intravenously with 0.5 cc. of citrated whole blood from a White Leghorn pullet with erythroleukosis. Unsuccessful attempts were made to inoculate the tumors by application of the material to scarified combs or by subcutaneous injection into White Leghorn cockerels. Crossbred chicks similar to those in which the tumor originated were not available for the inoculation experiments.—M. H. P.

Reticulum Cell Sarcoma of the Bone Marrow of a Pig. Plummer, P. J. G. [Science Service, Dominion Dept. of Agric., Animal Dis. Research Inst., Hull, Quebec] *Canad. J. Comp. Med.*, **9**:254-257. 1945.

A report of a case is given and includes photomicrographs. Although the animal appeared healthy when it was slaughtered, the marrow was found by the butcher to be abnormal and a portion of the carcass was submitted for histological study. The tumor was present throughout the marrow of all bones examined, including the terminal coccygeal vertebrae. The spleen, lymph glands, and blood were not available for histological study, but the butcher reported that the thoracic lymph gland was enlarged and the spleen was very firm.—M. H. P.

An Outline of Recent Advances in the Experimental Production of Tumours. Willis, R. A. [Dept. of Path., Alfred Hosp., Melbourne, Australia] *M. J. Australia*, **1**:361-368. 1945.

A review.—E. L. K.

Clinical and Pathological Reports

Clinical Investigations Are Sometimes Included Under Reports of Research

Diagnosis—General

Differential Diagnosis between Ruptured Lumber Intervertebral Disk and Certain Diseases of the Spinal and Peripheral Nervous Systems. Ray, B. S. [Cornell Univ. Med. Coll., New York, N. Y.] *S. Clin. North America*, **26**:272-281. 1946.

In this discussion the author considers the differential diagnosis between ruptured lumbar disk and intradural neoplasm. Three case histories are presented dealing with this part of the paper. Briefly mentioned are the intraspinal extradural tumors.—J. L. M.

The Differential Diagnosis of Splenomegaly of Adults. Hargraves, M. M. [Mayo Clinic, Rochester, Minn.] *M. Clin. North America*, **26**:731-797. 1946.

The relationship of leukemia to the cause of splenomegaly is discussed. Illustrative case histories are presented including those of patients with chronic myelogenous leukemia, chronic lymphatic leukemia, leukopenic myelogenous leukemia. Other causes of splenomegaly which are discussed, each with case reports, include lymphoblastoma, agnogenic myeloid metaplasia of the spleen, splenic lymphangiohemangioma, leiomyosarcoma, and hypernephroma. —J. L. M.

The Value of the Weltmann Serum Coagulation Reaction for the Diagnosis of Certain Forms of Malignant Neoplastic Disease. Wachstein, M. [Elizabeth A. Horton Memorial Hosp., Middletown, N. Y., and Beth Israel Hosp., Passaic, N. J.] *J. Lab. & Clin. Med.*, **30**:14-19. 1945.

The results of the Weltmann serum coagulation tests in 128 cases are tabulated. The author states in his discussion that the coagulation band is a non-specific reaction, both lobar pneumonia and nephrosis, for example, showing a pronounced left shift. He finds that the reaction shows a pronounced left shift in the large majority of patients with neoplastic disease and concludes that the test has differential diagnostic value.—J. G. K.

Early Diagnosis in Tumors of the Central Nervous System. Reid, W. L. [Sydney, Australia] *M. J. Australia*, **1**:865-872. 1946.

A summary and discussion of diagnostic methods—E. L. K.

A Test for Carcinoma. Robertson, F. N. [Vancouver, B. C.] *Canad. M. A. J.*, **51**:54-59. 1944.

A simple test on the urine and blood of cancer patients is described. One to 4 oz. of an early morning urine specimen should be tested for calcium by Sulkowitches reagent. Lime water is added until the calcium content is normal. The specimen is then transferred to an enamel kidney basin. Blood from the patient, about 4.0 cc., is then added to the urine in such a way that the blood slowly covers the bottom of the dish and the urine covers the blood. If clotting of the blood occurs, the dish should be gently agitated. A positive test is read when small clots cling to the bottom of the dish and are not dislodged by washing with cold water. The test has proved accurate in about 92% of 122 cases when tried among 5 independent workers at the Vancouver General Hospital.—M. E. H.

A Tentative Test For Pheochromocytoma. Roth, G. M., and Kvale, W. F. [Mayo Clinic, Rochester, Minn.] *Am. J. M. Sc.*, **210**:653-660. 1945.

When histamine base was given to 3 patients who had pheochromocytoma, the blood pressure rose approximately 100 mm. more than the elevation obtained by the cold pressor tests. This elevation was accompanied by the characteristic symptoms of a spontaneous attack. The authors conclude that the test may be considered tentatively as a worthwhile procedure in distinguishing the syndrome of pheochromocytoma from other conditions.—J. G. K.

Diagnosis and Description of Cancer. Harvey, W. F. [Roy. Coll. of Physicians, Edinburgh, Scotland] *Edinburgh M. J.*, **52**:181. 1945.

A discussion is given of various factors which are concerned in the histological diagnosis of cancer, based upon examination in the author's laboratory of 19,296 tumors. Cancer cells have certain recognizable features, i.e., hyperchromatism, mitotic activity, cell enlargement, alteration of cytoplasmic staining and so on, but these are not invariable. The diagnosis of cancer is made mainly and finally by tissue pattern and not from single cells.—E. L. K.

Therapy—General

Tumor Dosage and Results in Roentgen Therapy of Cancer of the Breast. Lenz, M. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Am. J. Roentgenol.*, **56**:67-74. 1946.

The histories of 206 patients with cancer of the breast who received roentgen therapy at the Presbyterian Hospital, New York, and of an unstated number of cases with remote metastasis treated at the Montefiore Hospital, New York, were studied in an attempt to correlate the results of therapy with the tumor dosage administered. In the period 1923 to 1929, a series of 78 patients received low doses of roentgen therapy postoperatively in repeated courses over several months. This treatment did not appear to influence the course of the disease, and in 8 instances, skin metastases developed in a previously irradiated area, indicating clearly the inadequacy of the dosage. Subsequently, the author abandoned routine postoperative irradiation, and now advocates such treatment only in cases where surgical removal of the breast tumor is known to have been incomplete. In the years 1933 to 1937, higher tumor doses ranging up to 4,500 r were delivered by fractionated irradiation, cross-firing the breast and axilla. The local disappearance of the primary tumor after irradiation did not influence the course of the disease in patients with distant metastasis. Local regression was observed more frequently when the tumors were of small size. Recognizable tumor cells were found on histological examination of all mastectomy specimens in this group. The author concluded that such doses are too low to destroy cancer of the breast, and accordingly, most of the patients treated since 1938 have received tumor doses of 5,500-8,000 r, delivered through multiple portals to the breast and axilla. There were 47 patients whose tumors, though localized to the breast and axilla, presented one or more of the stated criteria of inoperability, and roentgen therapy alone was employed in these cases. Gross persistence of cancer was noted in all primary sites receiving a tumor dose of less than 5,500 roentgens. By contrast, only 8 of 32 patients who received tumor doses over 5,500 r had grossly detectable residual disease in the breast. Of 24 patients whose tumors disappeared clinically after treatment, 11 were clinically free of cancer after 5 years. Since none of these patients was subsequently mastectomized, histological studies in this group are not available.

The author concludes that radical mastectomy is still the treatment of choice in operable cases of cancer of the breast. Roentgen therapy alone, in tumor doses over 6,000 r, is to be preferred to combined mastectomy and preoperative or postoperative irradiation in cases initially considered inoperable. Roentgen therapy of metastases is necessarily limited in dosage and only temporary regression or restraint of growth can be affected. Such treatment is particularly recommended for the relief of pain due to skeletal metastasis. Treatment of pulmonary or hepatic metastases is seldom of value. In patients who have not passed the menopause, local treatment of bone metastasis may be supplemented by roentgen castration—H. S. K.

Basic Facts Regarding X-Ray and Radium Therapy. Quimby, E. H. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *J. Am. Med. Women's Assoc.*, **1**:4-7. 1946.

The process of radiation is described, followed by a brief discussion of radiation therapy as it should be applied to specific tumor sites. The author points out that this is a field for a specialist, and that the radiologist must watch his patient carefully throughout treatment, concluding that "radiation in proper hands and properly used is a powerful weapon; in the wrong hands it may be extremely dangerous."—E. B. B.

Roentgen Therapy of Pituitary Adenomas. Mufson, J. A., and Blankstein, S. S. [Mt. Sinai Hospital, Milwaukee, Wis.] *Wisconsin M. J.*, **45**:680-685. 1946.

In the 3 cases presented in this report, radiotherapy was the sole form of treatment. The cases were all of the chromophobe type. Headache, a prominent symptom in all, disappeared early and there was moderate to marked improvement in visual acuity. The argument for roentgen therapy is strengthened when the mortality statistics of the x-ray treated group is compared with those receiving surgical treatment.—M. E. H.

De l'augmentation considérable du rendement profond par irradiation roentgenthérapique "ascendante" des cancers utérins. [Considerable Increase of Yield by "Ascendant" Roentgenotherapy in Cancers of the Cervix Uteri.] Lambret, G., and Swyngedauw, J. [Anticancer Center of the North Region, Lille, France] *Bull. Assoc. franç. p. l'étude du cancer*, **30**: 130-137. 1942.

The tube is placed under the bed where the patient lies in dorsal or abdominal decubitus. The bed is maintained at 170 cm. from the floor. By using that technic, in both dorsal and abdominal positions, the cervix uteri is brought closer to the skin surface, through which the x-rays penetrate. The yield of the irradiation is increased by 30%.—R. J.

The Use of Radon Ointment in the Treatment of Late Irradiation Ulcers. Kirsh, D., Mahoney, J. F., and Pendergrass, E. P. [Univ. of Pennsylvania, Philadelphia, Pa.] *Am. J. M. Sc.*, **212**:395-403. 1946.

The authors treated 17 cases presenting 19 late irradiation ulcers with radon ointment, a relatively new therapeutic agent introduced in 1930. Twelve of the ulcers healed completely in periods varying from 3 to 50 weeks. Two ulcers with residual malignancy showed a good response but failed to heal completely.—M. T.

Capsules and Inserter for the Use of Radium Cells in the Treatment of Cancer of the Uterine Fundus. Campbell, L. A. [Saginaw, Mich.] *Am. J. Roentgenol.*, **56**:208-210. 1946.

This is a description of a new type of holder and inserter for the placement of multiple radium capsules in the uterine cavity. This instrument is designed to accommodate the standard radium-containing platinum cell, thus eliminating the need for special radium sources. The method of insertion with this instrument is briefly described.—H.S.K.

Role of Radiotherapy in the Treatment of Malignant Neoplasms. Hunt, H. B. [Omaha, Neb.] *Nebraska M. J.*, **30**:192-196. 1945.

A general discussion—M. H. P.

Progesterone Therapy in Uterine Fibromyoma. Goodman, A. L. [Endocrine Clinic, Joanna McClelland Memorial Hosp., Waco, Texas] *J. Clin. Endocrinol.*, **6**: 402-408. 1946.

Stimulated by the work of Lipschütz in which progesterone treatment prevented the induction (under estrogenic stimulation) of fibromyomas of the uterus in the guinea pig, Goodman injected large doses of progesterone (10 mgm. 3 to 6 times weekly) in 7 women who had clinical evidence to support a diagnosis of uterine myomas. The size of the uterus was judged to decrease. The results were reported with the expressed hope that other investigators who had more elaborate facilities would check other cases, similarly treated, to determine the actual effect of progesterone treatment on the fibromyomas.—J. B. H.

The Effect of Stilbestrol on the Testis and Breast of Patients Treated for Carcinoma of the Prostate Gland. Schwartz, M. [Portland, Oregon.] *Rocky Mt. M. J.*, **43**:643. 1946.

Biopsies of breast and testicular tissue were obtained in several cases before and at varying intervals after the administration of stilbestrol (369 to 1,700 mgm.) to patients with prostatic carcinoma. Stilbestrol was capable of producing proliferative change in the male breast and marked degenerative changes in the human testes.—M. E.H.

Methyl Testosterone and Surgical Castration in the Treatment of Carcinoma of the Breast. Boger, W. P., *J. Clin. Endocrinol.*, **6**:88-98. 1946.

A 27 year-old patient with mammary cancer and extensive skeletal metastases was oöphorectomized bilaterally June 30, 1944, and on the day following the operation was placed upon a course of daily oral treatment of 20 mgm. of methyltestosterone. The patient was relieved of pain, gained weight, became ambulatory, and showed roentgenographic evidence of extensive calcification of skeleton in areas of cancerous metastases. No menopause-like signs appeared. This relief persisted for 8 months until March, 1945, when pain and progression of the cancerous state reappeared. Death occured in June, 1945. —J. B. H.

Hormonal Treatment of Carcinoma of the Prostate Gland. Edwards, C. [Sidney, Australia] *M. J. Australia*, **1**:165-168. 1945.

A review.—E. L. K.

The Surgical Treatment of Some Lesions of the Lower Esophagus and Upper Stomach. Bradshaw, H. H., and O'Neill, J. F. [Bowman Gray Sch. of Med., and North Carolina Baptist Hosp., Winston-Salem, N. C.] *J. Thoracic Surg.*, **14**:187-194. 1945.

During the past 2½ years, 5 patients with cancer or ulcer of the lower esophagus and upper stomach were subjected to partial or total gastrectomy by the transthoracic route, with esophagogastric or esophagojejunal anastomosis. Two died shortly after the operation and 3 survived; final results are not yet known. In addition to these cases presented in detail, 56 others are mentioned in which transthoracic gastrectomy with esophagogastrostomy or esophagojejunostomy was performed for cancer, with an operative mortality of 46.6%. The transthoracic approach is superior to the abdominal approach in that it permits resection of growths that have extended up the esophagus, and reveals supradiaphragmatic metastases.—M. H. P.

Nitrogen Mustard Therapy. Studies on the Effect of Methyl-Bis (Beta-Choroethyl) Amine Hydrochloride on Neoplastic Diseases and Allied Disorders of the Hemopoietic System. Jacobson, L. O., Spurr, C. L., Barron, E. S. G., Smith, T., Lushbaugh, C., and Dick, G. F. [Univ. of Chicago, Chicago, Ill.] *J. A. M. A.*, **132**:263-271. 1946.

A series of 59 patients with neoplastic and allied diseases of the hemopoietic system have been treated with methyl-*bis* (beta-chloroethyl) amine hydrochloride. The diagnosis was confirmed by biopsy or sternal puncture in each instance. The diseases treated include 27 cases of Hodgkin's disease; 6 of lymphosarcoma; 2 sympathoblastoma; 2 multiple myeloma; 8 myelogenous leukemia; 1 acute and 7 chronic forms; 5 polycythemia rubra, and 9 lymphatic leukemia, 1 acute and 8 chronic forms The dosage was 0.1 mgm. per kgm. of body weight given in courses of 1 to 7 daily injections. Nausea and vomiting 3 to 4 hours after administration of the drug was the usual immediate toxic reaction. The rapidity and severity of the lymphopenia, neutropenia and thrombocytopenia which almost invariably occurs within the first 3 weeks is dependent on the sensitivity of the patient, the disease, and the total amount of the drug given. The hematologic changes observed in the peripheral blood are paralleled in the bone marrow, and there is a return to normal values after varying intervals. The clinical remissions vary from zero to 18 months. Encouraging results have largely been confined to Hodgkin's disease. Lymphadenopathy, splenomegaly, hepatomegaly usually regress rapidly; repeated courses have produced further remissions. In certain cases of Hodgkin's disease classified as roentgen ray-resistant, significant clinical remissions have been produced.—M. E. H.

The Management of Inoperable Cancer. Treves, N. [Memorial Hosp., New York. N. Y., and Meadowbrook Hosp., Hempstead, N. Y.] *West Virginia M. J.* **41**:208-217. 1945.

A review with bibliography for general practitioners is presented. The subjects discussed include cancer statistics, euthanasia, nursing care, chemotherapy (negative clinical results with heptaldehyde bisulfite and avidin). Supportive measures for the patient, treatment for nausea, and vomiting, as well as care of cutaneous ulcerations are considered. Relief of pain by hypnotics, narcotics, alcohol injections, calcium medication, and cobra venom are discussed. —M. H. P.

Things to Do if Cancer of the Stomach is Oftener to be Cured. Report of an Instructive Case. Alvarez, W. C. [Mayo Clinic, Rochester, Minn.] *Gastroenterology*, **6**:574-575. 1946.

A case report of an adenocarcinoma of the pylorus in a 76 year old man is given. The author points out several lessons to be learned from this case: (1) a brief history of gastric "ulcer" in patients past middle age usually indicates cancer; (2) gastric analysis is of little diagnostic help; (3) disappearance of the ulcer crater under medical man-

agement "should not lead to any relaxation of vigilance." —H. H.

Principles Involved in Surgical Therapy of "Encapsulated" Connective Tissue Malignancies. Wolfer, J. A., and Carroll, W. W. [Chicago, Ill.] *Proc. Inst. Med. Chicago*, **16**:231-232. 1946.

The low rate of cure seems to be dependent on the extent of local excision at the time of the first surgical intervention. The author advises more radical early surgery.—M. E. H.

Skin and Subcutaneous Tissues

The Plastic Correction of Superficial Vascular and Pigmented Nevi. Greeley, P. W. [Univ. of Illinois Coll. of Med., Illinois Research and Educational Hosps., and St. Luke's Hosp., Chicago, Ill.] *Surgery*, **19**:467-481. 1946.

The surgical excision of 17 hemangiomas and nevi, in patients ranging from 1 to 36 years of age, is discussed. Many of these patients had had previous dermatologic treatment, which is of aid with smaller vascular nevi.—W.A.B.

Tumors of the Subcutaneous Tissue and Fascia. With Special Reference to Fibrosarcoma—A Clinical Study. Wilson, D. A. [Duke Univ. Sch. of Med., and Duke Hosp., Durham, N. C.] *Surg., Gynec., Obst.*, **80**:500-508. 1945.

From 80,000 surgical specimens examined during the years 1931 to 1941, 871 soft tissue tumors were classified, first according to the sex, race, and age of the patient, and second according to the anatomical distribution and size of the growth. There were 111 fibrosarcomas, 142 fibromas, 358 lipomas, 142 hemangiomas, 17 lymphangiomas, 39 angioendotheliomas, 31 neurofibromas, and 31 neurilemmomas. The ratio of benign to malignant tumors was 8 to 1.—J. G. K.

Sur trois cas de tumeurs sous-cutanées multiples. [Description of Three Cases of Multiple Subcutaneous Tumors.] Bablet, J., and Souchard, L. [Pasteur Inst., Paris, France] *Bull. Assoc. franç. p. l'étude du cancer*, **31**:189-194. 1943.

These 3 cases concern different malignant tumors which appeared in aged patients. These growths had the common characteristic of spreading early to the skin from subcutaneous tissue.—R. J.

Malignant Tumors of the Scalp. Figi, F. A. [Mayo Clinic, Rochester, Minn.] *S. Clin. North America*, **26**:859-870. 1946.

The most common primary malignant tumors of the scalp are basal cell and squamous cell epitheliomas. The latter usually develop on the cutaneous surface but they may originate within sebaceous cysts. Other primary neoplasms of the scalp include melanoepithelioma, fibrosarcoma, and lymphoblastoma (Hodgkin's disease). With the exception of the latter, surgical removal supplemented with radiation is the treatment of choice. Secondary malignant tumors of the scalp include metastatic hypernephromas, carcinomas and sarcoma. The carcinomas usually are primary in the stomach, colon, breast, bronchus or prostate. Multiple myelomas may also involve the scalp secondarily.—J. L. M.

An Enormous Epithelioma Adenoides Cysticum of the Scalp. Gabarro, P. [E.M.S. Hosp., England] With a Pathological Report by S. L. Baker [Univ. of Manchester, Manchester, England] *Brit J. Surg.*, **33**:188-190. 1945.

The first tumors had appeared 33 years before in the region of the right eye. During the last few months before operation the growths had enlarged to a turban-like mass covering the whole upper part of the head, and neoplastic lesions appeared on the trunk at the same time. All of the tumors had a strong smell of butyric acid. There was no familial history.—E. L. K.

A Case of Acanthosis Nigricans. O'Donnell, F. J., and O'Conner, M. H., *Irish J. M. Sc.*, **7**:163-164. 1944.

Description of a case.—E. L. K.

Nervous System

A Clinico-Pathological Study of Eight Cases of Medullo-Blastoma and of one of Spongioblastoma Ependymale Simulating Medulloblastoma. Swan, C. [Inst. of Med. and Veterinary Sc., Adelaide, Australia] *M. J. Australia*, **2**:153-160. 1944.

Of 8 patients suffering from medulloblastoma, 6 were males and 2 were females; the average age on admission to the hospital was 19.4 years, and the average duration of symptoms prior to admission was 4.9 months. A case of spongioblastoma ependymale is described, the patient being a female aged 13 years; the duration of symptoms prior to her admission to the hospital was 3 weeks. Medulloblastomas are twice as common in males as in females, not 3 times as common, as Cushing states. With one exception, symptoms and signs of increased intracranial pressure always preceded those due to dysfunction of the cerebellum and adjacent structures. Papilloedema is not necessarily a concomitant of increased intracranial pressure. X-ray examination of the skull was of assistance as a diagnostic measure in 4 out of 7 cases. The pressure of the cerebrospinal fluid was raised in 3 out of 5 cases in which it was estimated; the composition of the fluids showed no significant changes. Cerebellar dysfunction manifested itself most commonly as muscular incoordination associated with atonia and asthenia. Vertigo occurred in 5 cases and nystagmus in 4.

The average survival period in 4 cases of medulloblastoma, in which treatment was by partial or radical excision followed by deep x-ray therapy, was 10.1 months. The importance of repeated postoperative irradiation of the entire cerebrospinal axis is emphasized. The commonest causes of death were increased intracranial pressure (4 cases) and meningitis (3 cases).

The nature and origin of the cells of medulloblastomas are discussed. Variations in the cellularity, the vascularity, the amount of stroma and the number of mitoses did not appear to be of value in the determination of the grade of malignancy in the 8 cases of medulloblastoma studied. Local necrosis with involvement of blood vessels may be a factor leading to increase of collagen in medulloblastoma. Metastases were detected in only 1 of 5 cases of medulloblastoma in which autopsy was performed. Postmortem examinations, however, were confined to the cranial cavity. There was suggestive clinical evidence of

spinal metastases in one case. In comparison with those of medulloblastomas, the principal cells of the spongioblastoma ependymale studied were larger and rounder and had a more vesicular nucleus. The tumor was much more vascular and the vessels were considerably larger. The tumor cells had a pronounced tendency to arrange themselves around vessels. Connective tissue stroma was almost entirely absent. It is suggested that other examples of spongioblastoma ependymale have been classified erroneously as medullobastoma. Author's summary. (E. L. K.)

Neuroblastoma with Recovery. [Melbourne Pediat. Soc., Children's Hosp., Carlton, Melbourne, Australia] *M. J. Australia*, **2**:196-197. 1944.

A very large intraperitoneal cystic growth was removed successfully from a girl aged 4½ years.—E. L. K.

Cerebro-Spinal Fluid Protein and Intracranial Tumours. PHILLIPS, G., and GOSWELL, G. [Sydney, and Royal Australian Air Force, Australia] *M. J. Australia*, **1**:390-392. 1944.

The protein of the cerebro-spinal fluid was examined in a series of 161 intracranial tumors (gliomas, meningiomas, infratentorial tumors, tumors of the pituitary region, and others). The highest normal level is considered to be 40 mgm. per cent; 42 of the 161 cases showed greater amounts. Of 22 tumors in the cerebellum, 2 only produced a slight increase. The frequency of increase is greatest with the glioblastoma but in 66% of the cases of such tumor no change was observed. There is no constant relation between the histological type of tumor and increase in protein.—E. L. K.

EYE

Inheritance of Retinoblastoma. Two Families Supplying Evidence. FALLS, H. F. [Ann Arbor, Michigan] *J. A. M. A.*, **133**:171-174. 1947.

Retinoblastoma occurred bilaterally in identical twins in one family; in a second family both the horizontal and the vertical occurrence of retinoblastoma was observed. It is suggested that parents who have produced a child with retinoblastoma should be urged to discontinue having children and that children surviving enucleation for retinoblastoma should be sterilized.—M. E. H.

Malignant Lymphocytic Tumors of the Orbit. BENEDICT, W. L., and MARTENS, T. G. [Mayo Clinic, Rochester, Minn.] *S. Clin. North America*, **26**:871-875. 1946.

Malignant lymphocytic tumors situated in the orbit, as well as elsewhere, present problems of diagnosis, both clinically and histologically. Nineteen cases of primary lymphosarcoma of the orbit are considered, and brief reference is made to some other lymphogenous tumors of the region. One case in which the intraorbital and intracranial manifestations presented a difficult problem of diagnosis has been described.—J. L. M.

Treatment of Bilateral Retinoblastoma. DE ROETTH, A. F. [Spokane, Wash.] *Northwest Med.*, **43**:364-365. 1944.

Bilateral retinoblastoma in a 4 year old girl was successfully treated by removal of one eye and irradiation of the other by the method of Martin and Reese [*Arch. Ophth.*, **61**:733. 1936], employing the principle of the fractionated roentgen irradiation. Two cases treated by the author in Budapest with the electrocoagulation method of Weve [*Am. J. Ophth.*, **18**:575. 1935] are briefly mentioned; in 1 case the tumor was arrested for a few months only, in the other it was apparently cured (18 months follow-up). The author considers that the method of Martin and Reese surpasses all other methods of radiation treatment, and that the method of Weve has not yet been tried in a large enough series of cases to know its relative value. Because of the hereditary factor in retinoblastoma, and because of the need for early therapy, he urges that patients with this disease have their children's eyes examined in the first weeks of life and repeatedly thereafter. —M. H. P.

BREAST

Latent Primary Breast Tumour. GAHA, T. R. [Hobart, Tasmania, Australia] *M. J. Australia*, **1**:590. 1946.

There was considerable enlargement of an axillary gland, but no palpable breast tumor. After excision of the breast a small area of malignant duct papilloma was found in it, of the same type as the growth in the axillary gland. —E. L. K.

The Diagnosis of Tumors of the Breast. MARTIN, S. J. [Montreal Gen. Hosp., Montreal, Quebec] *S. Clin. North America*, **26**:319-325. 1946.

A general discussion is given under the following headings: examination of the breast, chronic cystic mastitis, sanguineous discharge from the nipple, benign tumors of the breast, cancer of the breast, and Paget's disease of the nipple.—J. L. M.

Brodie's Disease of the Breast AIKEN, D. [Christie Hosp., and Holt Radium Inst., Manchester, England] *Brit. J. Surg.*, **34**:87-90. 1946.

A woman aged 68 had a lump in her right breast. Since the patient's 28th year and during successive pregnancies, the mass had enlarged until removal, at which time it weighed 23 kgm. The tumor was exceedingly vascular and appeared to be a nonmalignant fibroadenoma.—E. L. K.

Adeno-Lipoma and Lipoma of the Breast. SPALDING, J. E. [Guy's Hosp., London, England] *Guy's Hosp. Rep.*, **94**:80-84. 1945.

Three kinds of fatty tumors of the breast may be distinguished, namely: (1) intramammary lipomas, arising in the fat of the intralobular connective tissue, (2) adenolipomas, mixed tumors of fat and epithelium, arising within the mammary lobule, and (3) paramammary lipomas, arising in the retromammary or subcutaneous fat but clinically involving the breast. An example of each of these varieties is described and illustrated.—E. L. K.

The Development of the Surgical Treatment of Carcinoma of the Breast. CRAIG, C., and HOLMAN, W. P. [Launceston General Hosp., Australia] **2**:201-205. 1944.

An historical review divided under the heading of 6 periods of which the first extends up to 1860 and the last from 1904 onwards.—E. L. K.

Modern Trends in the Treatment of Carcinoma of the Breast. CHANCE, O., *Irish J. M. Sc.*, **1**:105-110. 1944.

A review of 104 cases treated at St. Anne's Hospital is given. Keynes' method of applying radium after amputation is advocated.—E. L. K.

Book Review

CURRENTS IN BIOCHEMICAL RESEARCH. Edited by David E. Green. New York: Interscience Publishers, Inc. 1946, 486 pages. Price $5.00.

Contents: *The Gene and Biochemistry*, G. W. Beadle. *Viruses*, W. M. Stanley; *Photosynthesis and the Production of Organic Matter on Earth*, H. Gaffron; *The Bacterial Cell*, René J. Dubos; *The Nutrition and Biochemistry of Plants*, D. R. Hoagland; *Biological Significance of Vitamins*, C. A. Elvehjem; *Some Aspects of Vitamin Research*, Karl Folkers; *Quantitative Analysis in Biochemistry*, Donald D. Van Slyke; *Enzymic Hydrolysis and Synthesis of Peptide Bonds*, Joseph S. Fruton; *Metabolic Process Patterns*, Fritz Lippmann; *Biochemistry from the Standpoint of Enzymes*, David E. Green; *Enzymic Mechanisms of Carbon Dioxide Assimilation*, Severo Ochoa; *Hormones*, B. A. Houssay; *Fundamentals of Oxidation and Reduction*, Leonor Michaelis; *Mesomeric Concepts in the Biological Sciences*, Herman M. Kalckar; *Viscometry in Biochemical Investigations*, Max A. Lauffer; *Isotope Technique in the Study of Intermediary Metabolism*, D. Rittenberg and David Shemin; *Mucolytic Enzymes*, Karl Meyer; *Some Aspects of Intermediary Metabolism*, Konrad Bloch; *The Steroid Hormones*, Gregory Pincus; *Plant Hormones and the Analysis of Growth*, Kenneth V. Thimann; *Chemical Mechanisms of Nervous Action*, David Nachmansohn; *Some Aspects of Biochemical Antagonism*, D. W. Woolley; *Chemotherapy*, Rollin D. Hotchkiss; *Biochemical Aspects of Pharmacology*, Arnold D. Welch and Ernest Bueding. *Some Biochemical Problems Posed by a Disease of Muscle*, Charles L. Hoagland; *Physiology and Biochemistry*, Surgeon Captain C. H. Best; *X-Ray Diffraction and the Study of Fibrous Proteins*, I. Fankuchen and H. Mark; *Immunochemistry*, Michael Heidelberger; *Social Aspects of Nutrition*, W. H. Sebrell; *Organization and Support of Science in the United States*, L. C. Dunn.

The thirty-one essays of this volume are intended to provide, both for the working scientist and for intelligent (and fairly well-grounded) laymen, a general picture of present-day ideas, activities, and objectives in biochemistry. A glance at the foregoing table of contents at once reveals the manifold aspects of the field and discovers the best possible reason for the making of this book. The multitudinous lines of growth in biochemistry cannot all be known in adequate detail or grasped in decent perspective by a single person preoccupied with his own work. Dr. Green and his skillful collaborators must be thanked for their stimulating service of information and new insights. The editor is also to be commended for the powers of persuasion and perseverance that he must have exercised in realizing this work.

A detailed review of *Currents in Biochemical Research* is hardly possible. The authors of these essays speak handsomely for themselves, and they are all workers who deserve, and reward, serious attention. It may, however, be appropriate to offer the reflections of one reader on the book as a whole. The first impression, gained from the table of contents, is of looking into a kaleidoscope. In the course of reading, one or two unifying themes begin to appear. Of these, the most important is a renewed impression of the wide dominion of the enzyme in biochemical thought. Most of these essays are concerned with one or another relationship of biological catalysts: the gene as originator, governor, or determinant of specific enzymes; the enzyme-substrate relationship as reflected in anti-vitamins and anti-metabolites, the explicit action of drugs, and the design of chemotherapeutic agents; the regulation of enzyme systems, both from the point of view of the logistics of metabolic transformations and energy interchanges and from the point of view of the action of hormones and trace substances; the implications of enzyme-catalized, reversible, inter-related reactions for the dynamic steady states of metabolism (which is the important lesson dramatized by the application of isotopes to the study of intermediary metabolism); enzymatic mechanism of biological synthesis; the enzyme as a biochemical tool. These are constantly recurring patterns in a multitude of different fields.

Another unifying element is the generally conservative tone of most of these essays. The problems in hand, the available tools both old and new, and the directions of current investigations, are outlined with sober confidence. An impression is often gained of a powerful, steady and workmanlike application of method and principle leading in a short time to very great development of knowledge within the field. There is not as much of the spirit of intellectual play and of the entertainment of untried ideas and principles as there is, for instance, in the comparable *Perspectives in Biochemistry*, published in England 10 years ago. Perhaps the contemporary development of new and powerful research tools, the fascinations of rapid discovery, and the new accessibility to solutions of long-standing problems may temporarily embarrass the exercise of speculative imagination. Since this is clearly an era of rapid extension of the methods of biochemistry into many fields it may be as well to allow the new facts and relationships to speak for themselves, so that both old and new principles may be examined without prejudice in a fresh context.

Finally, these essays eloquently affirm the fundamental contributions of organic chemistry and of physics to the phenomenal development of modern biochemistry. It may be worth remembering that the degree of understanding of biological systems which makes possible the development of biochemical tools of research has been attained largely by the painstaking applications of methods and principles derived from these primary fields of study.

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